

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : C07H 21/04, C07K 1/00, 14/00, C12N 1/21, 15/00, 15/09, 15/63, 15/70, C12P 19/34	A1	(11) International Publication Number: WO 00/52027 (43) International Publication Date: 8 September 2000 (08.09.00)
(21) International Application Number: PCT/US00/05432 (22) International Filing Date: 2 March 2000 (02.03.00) (30) Priority Data: 60/122,389 2 March 1999 (02.03.99) US 60/126,049 23 March 1999 (23.03.99) US 60/136,744 28 May 1999 (28.05.99) US (71) Applicant: LIFE TECHNOLOGIES, INC. [US/US]; 9800 Medical Center Drive, Rockville, MD 20850 (US). (72) Inventors: HARTLEY, James, L.; 7409 Hillside Drive, Frederick, MD 21702 (US). BRASCH, Michael, A.; 20931 Sunnycres Road, Gaithersburg, MD 20882 (US). TEMPLE, Gary, F.; 114 Ridge Road, Washington Grove, MD 20882 (US). CHEO, David; 2006 Baltimore Road, #21, Rockville, MD 20851 (US). (74) Agents: ESMOND, Robert, W. et al.; Sterne, Kessler, Goldstein & Fox P.L.L.C., Suite 600, 1100 New York Avenue, N.W., Washington, DC 20005-3934 (US).		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>With an indication in relation to deposited biological material furnished under Rule 13bis separately from the description.</i>
(54) Title: COMPOSITIONS AND METHODS FOR USE IN RECOMBINATIONAL CLONING OF NUCLEIC ACIDS		
(57) Abstract <p>The present invention relates generally to compositions and methods for use in recombinational cloning of nucleic acid molecules. In particular, the invention relates to nucleic acid molecules encoding one or more recombination sites or portions thereof, to nucleic acid molecules comprising one or more of these recombination site nucleotide sequences and optionally comprising one or more additional physical or functional nucleotide sequences. The invention also relates to vectors comprising the nucleic acid molecules of the invention, to host cells comprising the vectors or nucleic acid molecules of the invention, to methods of producing polypeptides using the nucleic acid molecules of the invention, and to polypeptides encoded by these nucleic acid molecules or produced by the methods of the invention. The invention also relates to antibodies that bind to one or more polypeptides of the invention or epitopes thereof. The invention also relates to the use of these compositions in methods for recombinational cloning of nucleic acids, <i>in vitro</i> and <i>in vivo</i>, to provide chimeric DNA molecules that have particular characteristics and/or DNA segments.</p>		

BEST AVAILABLE COPY

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakhstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

Compositions and Methods for Use in Recombinational Cloning of Nucleic Acids

5

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates generally to recombinant DNA technology. More particularly, the present invention relates to compositions and methods for use in recombinational cloning of nucleic acid molecules. The invention relates specifically to nucleic acid molecules encoding one or more recombination sites or one or more partial recombination sites, particularly *attB*, *attP*, *attL*, and *attR*, and fragments, mutants, variants and derivatives thereof. The invention also relates to such nucleic acid molecules wherein the one or more recombination site nucleotide sequences is operably linked to the one or more additional physical or functional nucleotide sequences. The invention also relates to vectors comprising the nucleic acid molecules of the invention, to host cells comprising the vectors or nucleic acid molecules of the invention, to methods of producing polypeptides and RNAs encoded by the nucleic acid molecules of the invention, and to polypeptides encoded by these nucleic acid molecules or produced by the methods of the invention, which may be fusion proteins. The invention also relates to antibodies that bind to one or more polypeptides of the invention or epitopes thereof, which may be monoclonal or polyclonal antibodies. The invention also relates to the use of these nucleic acid molecules, vectors, polypeptides and antibodies in methods for recombinational cloning of nucleic acids, *in vitro* and *in vivo*, to provide chimeric DNA molecules that have particular characteristics and/or DNA segments. More particularly, the antibodies of the invention may be used to identify and/or purify proteins or fusion proteins encoded by the nucleic acid molecules or vectors of the invention, or to identify and/or purify the nucleic acid molecules of the invention.

Related Art

Site-specific recombinases. Site-specific recombinases are proteins that are present in many organisms (e.g. viruses and bacteria) and have been characterized to have both endonuclease and ligase properties. These recombinases (along with associated proteins in some cases) recognize specific sequences of bases in DNA and exchange the DNA segments flanking those segments. The recombinases and associated proteins are collectively referred to as "recombination proteins" (see, e.g., Landy, A., *Current Opinion in Biotechnology* 3:699-707 (1993)).

Numerous recombination systems from various organisms have been described. See, e.g., Hoess *et al.*, *Nucleic Acids Research* 14(6):2287 (1986); Abremski *et al.*, *J. Biol. Chem.* 261(1):391 (1986); Campbell, J. *Bacteriol.* 174(23):7495 (1992); Qian *et al.*, *J. Biol. Chem.* 267(11):7794 (1992); Araki *et al.*, *J. Mol. Biol.* 225(1):25 (1992); Maeser and Kahnmann *Mol. Gen. Genet.* 230:170-176 (1991); Esposito *et al.*, *Nucl. Acids Res.* 25(18):3605 (1997).

Many of these belong to the integrase family of recombinases (Argos *et al.* *EMBO J.* 5:433-440 (1986); Voziyanov *et al.*, *Nucl. Acids Res.* 27:930 (1999)). Perhaps the best studied of these are the Integrase/*att* system from bacteriophage λ (Landy, A. *Current Opinions in Genetics and Devel.* 3:699-707 (1993)), the Cre/*loxP* system from bacteriophage P1 (Hoess and Abremski (1990) In *Nucleic Acids and Molecular Biology*, vol. 4. Eds.: Eckstein and Lilley, Berlin-Heidelberg: Springer-Verlag; pp. 90-109), and the FLP/FRT system from the *Saccharomyces cerevisiae* 2 μ circle plasmid (Broach *et al.* *Cell* 29:227-234 (1982)).

Backman (U.S. Patent No. 4,673,640) discloses the *in vivo* use of λ recombinase to recombine a protein producing DNA segment by enzymatic site-specific recombination using wild-type recombination sites *attB* and *attP*.

Hasan and Szybalski (*Gene* 56:145-151 (1987)) discloses the use of λ Int recombinase *in vivo* for intramolecular recombination between wild type *attP* and *attB* sites which flank a promoter. Because the orientations of these sites are

inverted relative to each other, this causes an irreversible flipping of the promoter region relative to the gene of interest.

Palazzolo *et al.* *Gene* 88:25-36 (1990), discloses phage lambda vectors having bacteriophage λ arms that contain restriction sites positioned outside a cloned DNA sequence and between wild-type *loxP* sites. Infection of *E. coli* cells that express the Cre recombinase with these phage vectors results in recombination between the *loxP* sites and the *in vivo* excision of the plasmid replicon, including the cloned cDNA.

Pósfai *et al.* (*Nucl. Acids Res.* 22:2392-2398 (1994)) discloses a method for inserting into genomic DNA partial expression vectors having a selectable marker, flanked by two wild-type FRT recognition sequences. FLP site-specific recombinase as present in the cells is used to integrate the vectors into the genome at predetermined sites. Under conditions where the replicon is functional, this cloned genomic DNA can be amplified.

Bebee *et al.* (U.S. Patent No. 5,434,066) discloses the use of site-specific recombinases such as Cre for DNA containing two *loxP* sites for *in vivo* recombination between the sites.

Boyd (*Nucl. Acids Res.* 21:817-821 (1993)) discloses a method to facilitate the cloning of blunt-ended DNA using conditions that encourage intermolecular ligation to a dephosphorylated vector that contains a wild-type *loxP* site acted upon by a Cre site-specific recombinase present in *E. coli* host cells.

Waterhouse *et al.* (WO 93/19172 and *Nucleic Acids Res.* 21 (9):2265 (1993)) disclose an *in vivo* method where light and heavy chains of a particular antibody were cloned in different phage vectors between *loxP* and *loxP 511* sites and used to transfect new *E. coli* cells. Cre, acting in the host cells on the two parental molecules (one plasmid, one phage), produced four products in equilibrium: two different cointegrates (produced by recombination at either *loxP* or *loxP 511* sites), and two daughter molecules, one of which was the desired product.

Schlake & Bode (*Biochemistry* 33:12746-12751 (1994)) discloses an *in vivo* method to exchange expression cassettes at defined chromosomal locations, each flanked by a wild type and a spacer-mutated FRT recombination site. A

double-reciprocal crossover was mediated in cultured mammalian cells by using this FLP/FRT system for site-specific recombination.

Hartley *et al.* (U.S. Patent No. 5,888,732) disclose compositions and methods for recombinational exchange of nucleic acid segments and molecules, including for use in recombinational cloning of a variety of nucleic acid molecules *in vitro* and *in vivo*, using a combination of wildtype and mutated recombination sites and recombination proteins.

Transposases. The family of enzymes, the transposases, has also been used to transfer genetic information between replicons. Transposons are structurally variable, being described as simple or compound, but typically encode the recombinase gene flanked by DNA sequences organized in inverted orientations. Integration of transposons can be random or highly specific. Representatives such as Tn7, which are highly site-specific, have been applied to the *in vivo* movement of DNA segments between replicons (Lucklow *et al.*, *J. Virol.* 67:4566-4579 (1993)).

Devine and Boeke *Nucl. Acids Res.* 22:3765-3772 (1994), discloses the construction of artificial transposons for the insertion of DNA segments, *in vitro*, into recipient DNA molecules. The system makes use of the integrase of yeast TY1 virus-like particles. The DNA segment of interest is cloned, using standard methods, between the ends of the transposon-like element TY1. In the presence of the TY1 integrase, the resulting element integrates randomly into a second target DNA molecule.

Recombination Sites. Also key to the integration/recombination reactions mediated by the above-noted recombination proteins and/or transposases are recognition sequences, often termed "recombination sites," on the DNA molecules participating in the integration/recombination reactions. These recombination sites are discrete sections or segments of DNA on the participating nucleic acid molecules that are recognized and bound by the recombination proteins during the initial stages of integration or recombination. For example, the recombination site for Cre recombinase is *loxP* which is a 34 base pair sequence comprised of two 13 base pair inverted repeats (serving as the recombinase binding sites) flanking an 8 base pair core sequence. See Figure 1 of Sauer, B., *Curr. Opin. Biotech.*

5:521-527 (1994). Other examples of recognition sequences include the *attB*,
attP, *attL*, and *attR* sequences which are recognized by the recombination protein
 λ Int. *attB* is an approximately 25 base pair sequence containing two 9 base pair
core-type Int binding sites and a 7 base pair overlap region, while *attP* is an
5 approximately 240 base pair sequence containing core-type Int binding sites and
arm-type Int binding sites as well as sites for auxiliary proteins integration host
factor (IHF), FIS and excisionase (Xis). See Landy, *Curr. Opin. Biotech.*
3:699-707 (1993); see also U.S. Patent No. 5,888,732, which is incorporated by
reference herein.

10 **DNA cloning.** The cloning of DNA segments currently occurs as a daily
routine in many research labs and as a prerequisite step in many genetic analyses.
The purpose of these clonings is various, however, two general purposes can be
considered: (1) the initial cloning of DNA from large DNA or RNA segments
(chromosomes, YACs, PCR fragments, mRNA, etc.), done in a relative handful
15 of known vectors such as pUC, pGem, pBlueScript, and (2) the subcloning of
these DNA segments into specialized vectors for functional analysis. A great deal
of time and effort is expended both in the transfer of DNA segments from the
initial cloning vectors to the more specialized vectors. This transfer is called
subcloning.

20 The basic methods for cloning have been known for many years and have
changed little during that time. A typical cloning protocol is as follows:

- (1) digest the DNA of interest with one or two restriction enzymes;
- (2) gel purify the DNA segment of interest when known;
- (3) prepare the vector by cutting with appropriate restriction
25 enzymes, treating with alkaline phosphatase, gel purify etc., as
appropriate;
- (4) ligate the DNA segment to the vector, with appropriate
controls to eliminate background of uncut and self-ligated vector;
- (5) introduce the resulting vector into an *E. coli* host cell;
- 30 (6) pick selected colonies and grow small cultures overnight;
- (7) make DNA minipreps; and

(8) analyze the isolated plasmid on agarose gels (often after diagnostic restriction enzyme digestions) or by PCR.

The specialized vectors used for subcloning DNA segments are functionally diverse. These include but are not limited to: vectors for expressing nucleic acid molecules in various organisms; for regulating nucleic acid molecule expression; for providing tags to aid in protein purification or to allow tracking of proteins in cells; for modifying the cloned DNA segment (*e.g.*, generating deletions); for the synthesis of probes (*e.g.*, riboprobes); for the preparation of templates for DNA sequencing; for the identification of protein coding regions; for the fusion of various protein-coding regions; to provide large amounts of the DNA of interest, *etc.* It is common that a particular investigation will involve subcloning the DNA segment of interest into several different specialized vectors.

As known in the art, simple subclonings can be done in one day (*e.g.*, the DNA segment is not large and the restriction sites are compatible with those of the subcloning vector). However, many other subclonings can take several weeks, especially those involving unknown sequences, long fragments, toxic genes, unsuitable placement of restriction sites, high backgrounds, impure enzymes, *etc.* Subcloning DNA fragments is thus often viewed as a chore to be done as few times as possible.

Several methods for facilitating the cloning of DNA segments have been described, *e.g.*, as in the following references.

Ferguson, J., *et al.* *Gene* 16:191 (1981), discloses a family of vectors for subcloning fragments of yeast DNA. The vectors encode kanamycin resistance. Clones of longer yeast DNA segments can be partially digested and ligated into the subcloning vectors. If the original cloning vector conveys resistance to ampicillin, no purification is necessary prior to transformation, since the selection will be for kanamycin.

Hashimoto-Gotoh, T., *et al.* *Gene* 41:125 (1986), discloses a subcloning vector with unique cloning sites within a streptomycin sensitivity gene; in a streptomycin-resistant host, only plasmids with inserts or deletions in the dominant sensitivity gene will survive streptomycin selection.

Accordingly, traditional subcloning methods, using restriction enzymes and ligase, are time consuming and relatively unreliable. Considerable labor is expended, and if two or more days later the desired subclone can not be found among the candidate plasmids, the entire process must then be repeated with alternative conditions attempted. Although site specific recombinases have been used to recombine DNA *in vivo*, the successful use of such enzymes *in vitro* was expected to suffer from several problems. For example, the site specificities and efficiencies were expected to differ *in vitro*; topologically linked products were expected; and the topology of the DNA substrates and recombination proteins was expected to differ significantly *in vitro* (see, e.g., Adams *et al*, *J. Mol. Biol.* 226:661-73 (1992)). Reactions that could go on for many hours *in vivo* were expected to occur in significantly less time *in vitro* before the enzymes became inactive. In addition, the stabilities of the recombination enzymes after incubation for extended periods of time in *in vitro* reactions was unknown, as were the effects of the topologies (*i.e.*, linear, coiled, supercoiled, etc.) of the nucleic acid molecules involved in the reaction. Multiple DNA recombination products were expected in the biological host used, resulting in unsatisfactory reliability, specificity or efficiency of subcloning. Thus, *in vitro* recombination reactions were not expected to be sufficiently efficient to yield the desired levels of product.

Accordingly, there is a long felt need to provide an alternative subcloning system that provides advantages over the known use of restriction enzymes and ligases.

SUMMARY OF THE INVENTION

The present invention relates to nucleic acid molecules encoding one or more recombination sites or one or more partial recombination sites, particularly *attB*, *attP*, *attL*, and *attR*, and fragments, mutants, variants and derivatives thereof. The invention also relates to such nucleic acid molecules comprising one or more of the recombination site nucleotide sequences or portions thereof and one or more additional physical or functional nucleotide sequences, such as those

encoding one or more multiple cloning sites, one or more transcription termination sites, one or more transcriptional regulatory sequences (*e.g.*, one or more promoters, enhancers, or repressors), one or more translational signal sequences, one or more nucleotide sequences encoding a fusion partner protein or peptide
5 (*e.g.*, GST, His₆ or thioredoxin), one or more selection markers or modules, one or more nucleotide sequences encoding localization signals such as nuclear localization signals or secretion signals, one or more origins of replication, one or more protease cleavage sites, one or more desired proteins or peptides encoded by a gene or a portion of a gene, and one or more 5' or 3' polynucleotide tails
10 (particularly a poly-G tail). The invention also relates to such nucleic acid molecules wherein the one or more recombination site nucleotide sequences is operably linked to the one or more additional physical or functional nucleotide sequences.

The invention also relates to primer nucleic acid molecules comprising the
15 recombination site nucleotide sequences of the invention (or portions thereof), and to such primer nucleic acid molecules linked to one or more target-specific (*e.g.*, one or more gene-specific) primer nucleic acid sequences. Such primers may also comprise sequences complementary or homologous to DNA or RNA sequences to be amplified, *e.g.*, by PCR, RT-PCR, etc. Such primers may also comprise
20 sequences or portions of sequences useful in the expression of protein genes (ribosome binding sites, localization signals, protease cleavage sites, repressor binding sites, promoters, transcription stops, stop codons, etc.). Said primers may also comprise sequences or portions of sequences useful in the manipulation of DNA molecules (restriction sites, transposition sites, sequencing primers, etc.).
25 The primers of the invention may be used in nucleic acid synthesis and preferably are used for amplification (*e.g.*, PCR) of nucleic acid molecules. When the primers of the invention include target- or gene-specific sequences (any sequence contained within the target to be synthesized or amplified including translation signals, gene sequences, stop codons, transcriptional signals (*e.g.*, promoters) and
30 the like), amplification or synthesis of target sequences or genes may be accomplished. Thus, the invention relates to synthesis of a nucleic acid molecules comprising mixing one or more primers of the invention with a nucleic acid

template, and incubating said mixture under conditions sufficient to make a first nucleic acid molecule complementary to all or a portion of said template. Thus, the invention relates specifically to a method of synthesizing a nucleic acid molecule comprising:

- (a) mixing a nucleic acid template with a polypeptide having polymerase activity and one or more primers comprising one or more recombination sites or portions thereof; and
- (b) incubating said mixture under conditions sufficient to synthesize a first nucleic acid molecule complementary to all or a portion of said template and which preferably comprises one or more recombination sites or portions thereof.

Such method of the invention may further comprise incubating said first synthesized nucleic acid molecule under conditions sufficient to synthesize a second nucleic acid molecule complementary to all or a portion of said first nucleic acid molecule. Such synthesis may provide for a first nucleic acid molecule having a recombination site or portion thereof at one or both of its termini.

In a preferred aspect, for the synthesis of the nucleic acid molecules, at least two primers are used wherein each primer comprises a homologous sequence at its terminus and/or within internal sequences of each primer (which may have a homology length of about 2 to about 500 bases, preferably about 3 to about 100 bases, about 4 to about 50 bases, about 5 to about 25 bases and most preferably about 6 to about 18 base overlap). In a preferred aspect, the first such primer comprises at least one target-specific sequence and at least one recombination site or portion thereof while the second primer comprises at least one recombination site or portion thereof. Preferably, the homologous regions between the first and second primers comprise at least a portion of the recombination site. In another aspect, the homologous regions between the first and second primers may comprise one or more additional sequences, *e.g.*, expression signals, translational start motifs, or other sequences adding functionality to the desired nucleic acid sequence upon amplification. In practice, two pairs of primers prime synthesis or amplification of a nucleic acid molecule. In a preferred aspect, all or at least a portion of the synthesized or amplified nucleic acid molecule will be homologous

to all or a portion of the template and further comprises a recombination site or a portion thereof at at least one terminus and preferably both termini of the synthesized or amplified molecule. Such synthesized or amplified nucleic acid molecule may be double stranded or single stranded and may be used in the recombinational cloning methods of the invention. The homologous primers of the invention provide a substantial advantage in that one set of the primers may be standardized for any synthesis or amplification reaction. That is, the primers providing the recombination site sequences (without the target specific sequences) can be pre-made and readily available for use. This in practice allows the use of shorter custom made primers that contain the target specific sequence needed to synthesize or amplify the desired nucleic acid molecule. Thus, this provides reduced time and cost in preparing target specific primers (e.g., shorter primers containing the target specific sequences can be prepared and used in synthesis reactions). The standardized primers, on the other hand, may be produced in mass to reduce cost and can be readily provided (e.g., in kits or as a product) to facilitate synthesis of the desired nucleic acid molecules.

Thus, in one preferred aspect, the invention relates to a method of synthesizing or amplifying one or more nucleic acid molecules comprising:

- (a) mixing one or more nucleic acid templates with at least one polypeptide having polymerase or reverse transcriptase activity and at least a first primer comprising a template specific sequence (complementary to or capable of hybridizing to said templates) and at least a second primer comprising all or a portion of a recombination site wherein said at least a portion of said second primer is homologous to or complementary to at least a portion of said first primer; and
- (b) incubating said mixture under conditions sufficient to synthesize or amplify one or more nucleic acid molecules complementary to all or a portion of said templates and comprising one or more recombination sites or portions thereof at one and preferably both termini of said molecules.

More specifically, the invention relates to a method of synthesizing or amplifying one or more nucleic acid molecules comprising:

- (a) mixing one or more nucleic acid templates with at least one polypeptide having polymerase or reverse transcriptase activity and at least a first primer comprising a template specific sequence (complementary to or capable of hybridizing to said templates) and at least a portion of a recombination site, and at least a second primer comprising all or a portion of a recombination site wherein said at least a portion of said recombination site on said second primer is complementary to or homologous to at least a portion of said recombination site on said first primer; and
- (b) incubating said mixture under conditions sufficient to synthesize or amplify one or more nucleic acid molecules complementary to all or a portion of said templates and comprising one or more recombination sites or portions thereof at one and preferably both termini of said molecules.

In a more preferred aspect, the invention relates to a method of amplifying or synthesizing one or more nucleic acid molecules comprising:

- (a) mixing one or more nucleic acid templates with at least one polypeptide having polymerase or reverse transcriptase activity and one or more first primers comprising at least a portion of a recombination site and a template specific sequence (complementary to or capable of hybridizing to said template);
- (b) incubating said mixture under conditions sufficient to synthesize or amplify one or more first nucleic acid molecules complementary to all or a portion of said templates wherein said molecules comprise at least a portion of a recombination site at one and preferably both termini of said molecules;
- (c) mixing said molecules with one or more second primers comprising one or more recombination sites, wherein said recombination sites of said second primers are homologous to or

complementary to at least a portion of said recombination sites on said first nucleic acid molecules; and

- (d) incubating said mixture under conditions sufficient to synthesize or amplify one or more second nucleic acid molecules complementary to all or a portion of said first nucleic acid molecules and which comprise one or more recombination sites at one and preferably both termini of said molecules.

The invention also relates to vectors comprising the nucleic acid molecules of the invention, to host cells comprising the vectors or nucleic acid molecules of the invention, to methods of producing polypeptides encoded by the nucleic acid molecules of the invention, and to polypeptides encoded by these nucleic acid molecules or produced by the methods of the invention, which may be fusion proteins. The invention also relates to antibodies that bind to one or more polypeptides of the invention or epitopes thereof, which may be monoclonal or polyclonal antibodies. The invention also relates to the use of these nucleic acid molecules, primers, vectors, polypeptides and antibodies in methods for recombinational cloning of nucleic acids, *in vitro* and *in vivo*, to provide chimeric DNA molecules that have particular characteristics and/or DNA segments.

The antibodies of the invention may have particular use to identify and/or purify peptides or proteins (including fusion proteins produced by the invention), and to identify and/or purify the nucleic acid molecules of the invention or portions thereof.

The methods for *in vitro* or *in vivo* recombinational cloning of nucleic acid molecule generally relate to recombination between at least a first nucleic acid molecule having at least one recombination site and a second nucleic acid molecule having at least one recombination site to provide a chimeric nucleic acid molecule. In one aspect, the methods relate to recombination between a first vector having at least one recombination site and a second vector having at least one recombination site to provide a chimeric vector. In another aspect, a nucleic acid molecule having at least one recombination site is combined with a vector having at least one recombination site to provide a chimeric vector. In a most preferred aspect, the nucleic acid molecules or vectors used in recombination

comprise two or more recombination sites. In a more specific embodiment of the invention, the recombination methods relate to a Destination Reaction (also referred to herein as an "LR reaction") in which recombination occurs between an Entry clone and a Destination Vector. Such a reaction transfers the nucleic acid molecule of interest from the Entry Clone into the Destination Vector to create an Expression Clone. The methods of the invention also specifically relate to an Entry or Gateward reaction (also referred to herein as a "BP reaction") in which an Expression Clone is recombined with a Donor vector to produce an Entry clone. In other aspects, the invention relates to methods to prepare Entry clones by combining an Entry vector with at least one nucleic acid molecule (e.g., gene or portion of a gene). The invention also relates to conversion of a desired vector into a Destination Vector by including one or more (preferably at least two) recombination sites in the vector of interest. In a more preferred aspect, a nucleic acid molecule (e.g., a cassette) having at least two recombination sites flanking a selectable marker (e.g., a toxic gene or a genetic element preventing the survival of a host cell containing that gene or element, and/or preventing replication, partition or heritability of a nucleic acid molecule (e.g., a vector or plasmid) comprising that gene or element) is added to the vector to make a Destination Vector of the invention.

Preferred vectors for use in the invention include prokaryotic vectors, eukaryotic vectors, or vectors which may shuttle between various prokaryotic and/or eukaryotic systems (e.g. shuttle vectors). Preferred prokaryotic vectors for use in the invention include but are not limited to vectors which may propagate and/or replicate in gram negative and/or gram positive bacteria, including bacteria of the genera *Escherichia*, *Salmonella*, *Proteus*, *Clostridium*, *Klebsiella*, *Bacillus*, *Streptomyces*, and *Pseudomonas* and preferably in the species *E. coli*. Eukaryotic vectors for use in the invention include vectors which propagate and/or replicate in yeast cells, plant cells, mammalian cells, (particularly human and mouse), fungal cells, insect cells, nematode cells, fish cells and the like. Particular vectors of interest include but are not limited to cloning vectors, sequencing vectors, expression vectors, fusion vectors, two-hybrid vectors, gene therapy vectors, phage display vectors, gene-targeting vectors, PACs, BACs, YACs, MACs, and

reverse two-hybrid vectors. Such vectors may be used in prokaryotic and/or eukaryotic systems depending on the particular vector.

In another aspect, the invention relates to kits which may be used in carrying out the methods of the invention, and more specifically relates to cloning or subcloning kits and kits for carrying out the LR Reaction (*e.g.*, making an Expression Clone), for carrying out the BP Reaction (*e.g.*, making an Entry Clone), and for making Entry Clone and Destination Vector molecules of the invention. Such kits may comprise a carrier or receptacle being compartmentalized to receive and hold therein any number of containers. Such containers may contain any number of components for carrying out the methods of the invention or combinations of such components. In particular, a kit of the invention may comprise one or more components (or combinations thereof) selected from the group consisting of one or more recombination proteins or auxiliary factors or combinations thereof, one or more compositions comprising one or more recombination proteins or auxiliary factors or combinations thereof (for example, GATEWAY™ LR Clonase™ Enzyme Mix or GATEWAY™ BP Clonase™ Enzyme Mix), one or more reaction buffers, one or more nucleotides, one or more primers of the invention, one or more restriction enzymes, one or more ligases, one or more polypeptides having polymerase activity (*e.g.*, one or more reverse transcriptases or DNA polymerases), one or more proteinases (*e.g.*, proteinase K or other proteinases), one or more Destination Vector molecules, one or more Entry Clone molecules, one or more host cells (*e.g.* competent cells, such as *E. coli* cells, yeast cells, animal cells (including mammalian cells, insect cells, nematode cells, avian cells, fish cells, etc.), plant cells, and most particularly *E. coli* DB3.1 host cells, such as *E. coli* LIBRARY EFFICIENCY® DB3.1™ Competent Cells), instructions for using the kits of the invention (*e.g.*, to carry out the methods of the invention), and the like. In related aspects, the kits of the invention may comprise one or more nucleic acid molecules encoding one or more recombination sites or portions thereof, particularly one or more nucleic acid molecules comprising a nucleotide sequence encoding the one or more recombination sites or portions thereof of the invention. Preferably, such nucleic acid molecules comprise at least two recombination sites which flank a selectable

marker (*e.g.*, a toxic gene and/or antibiotic resistance gene). In a preferred aspect, such nucleic acid molecules are in the form of a cassette (*e.g.*, a linear nucleic acid molecule comprising one or more and preferably two or more recombination sites or portions thereof).

5 Kits for inserting or adding recombination sites to nucleic acid molecules of interest may comprise one or more nucleases (preferably restriction endonucleases), one or more ligases, one or more topoisomerases, one or more polymerases, and one or more nucleic acid molecules or adapters comprising one or more recombination sites. Kits for integrating recombination sites into one or
10 more nucleic acid molecules of interest may comprise one or more components (or combinations thereof) selected from the group consisting of one or more integration sequences comprising one or more recombination sites. Such integration sequences may comprise one or more transposons, integrating viruses, homologous recombination sequences, RNA molecules, one or more host cells
15 and the like.

 Kits for making the Entry Clone molecules of the invention may comprise any or a number of components and the composition of such kits may vary depending on the specific method involved. Such methods may involve inserting the nucleic acid molecules of interest into an Entry or Donor Vector by the
20 recombinational cloning methods of the invention, or using conventional molecular biology techniques (*e.g.*, restriction enzyme digestion and ligation). In a preferred aspect, the Entry Clone is made using nucleic acid amplification or synthesis products. Kits for synthesizing Entry Clone molecules from amplification or synthesis products may comprise one or more components (or combinations
25 thereof) selected from the group consisting of one or more Donor Vectors (*e.g.*, one or more attP vectors including, but not limited to, pDONR201 (Figure 49), pDONR202 (Figure 50), pDONR203 (Figure 51), pDONR204 (Figure 52), pDONR205 (Figure 53), pDONR206 (Figure 53), and the like), one or more polypeptides having polymerase activity (preferably DNA polymerases and most preferably thermostable DNA polymerases), one or more proteinases, one or more
30 reaction buffers, one or more nucleotides, one or more primers comprising one or

more recombination sites or portions thereof, and instructions for making one or more Entry Clones.

Kits for making the Destination vectors of the invention may comprise any number of components and the compositions of such kits may vary depending on the specific method involved. Such methods may include the recombination methods of the invention or conventional molecular biology techniques (e.g., restriction endonuclease digestion and ligation). In a preferred aspect, the Destination vector is made by inserting a nucleic acid molecule comprising at least one recombination site (or portion thereof) of the invention (preferably a nucleic acid molecule comprising at least two recombination sites or portions thereof flanking a selectable marker) into a desired vector to convert the desired vector into a Destination vector of the invention. Such kits may comprise at least one component (or combinations thereof) selected from the group consisting of one or more restriction endonucleases, one or more ligases, one or more polymerases, one or more nucleotides, reaction buffers, one or more nucleic acid molecules comprising at least one recombination site or portion thereof (preferably at least one nucleic acid molecule comprising at least two recombination sites flanking at least one selectable marker, such as a cassette comprising at least one selectable marker such as antibiotic resistance genes and/or toxic genes), and instructions for making such Destination vectors.

The invention also relates to kits for using the antibodies of the invention in identification and/or isolation of peptides and proteins (which may be fusion proteins) produced by the nucleic acid molecules of the invention, and for identification and/or isolation of the nucleic acid molecules of the invention or portions thereof. Such kits may comprise one or more components (or combination thereof) selected from the group consisting of one or more antibodies of the invention, one or more detectable labels, one or more solid supports and the like.

Other preferred embodiments of the present invention will be apparent to one of ordinary skill in light of what is known in the art, in light of the following drawings and description of the invention, and in light of the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts one general method of the present invention, wherein the starting (parent) DNA molecules can be circular or linear. The goal is to exchange the new subcloning vector D for the original cloning vector B. It is desirable in one embodiment to select for AD and against all the other molecules, including the Cointegrate. The square and circle are sites of recombination: *e.g.*, *lox* (such as *loxP*) sites, *att* sites, *etc.* For example, segment D can contain expression signals, protein fusion domains, new drug markers, new origins of replication, or specialized functions for mapping or sequencing DNA. It should be noted that the cointegrate molecule contains Segment D (Destination vector) adjacent to segment A (Insert), thereby juxtaposing functional elements in D with the insert in A. Such molecules can be used directly in vitro (*e.g.*, if a promoter is positioned adjacent to a gene-for in vitro transcription/translation) or in vivo (following isolation in a cell capable of propagating *ccdB*-containing vectors) by selecting for the selection markers in Segments B+D. As one skilled in the art will recognize, this single step method has utility in certain envisioned applications of the invention.

Figure 2 is a more detailed depiction of the recombinational cloning system of the invention, referred to herein as the "GATEWAY™ Cloning System." This figure depicts the production of Expression Clones via a "Destination Reaction," which may also be referred to herein as an "LR Reaction." A *kan^r* vector (referred to herein as an "Entry clone") containing a DNA molecule of interest (*e.g.*, a gene) localized between an *attL1* site and an *attL2* site is reacted with an *amp^r* vector (referred to herein as a "Destination Vector") containing a toxic or "death" gene localized between an *attR1* site and an *attR2* site, in the presence of GATEWAY™ LR Clonase™ Enzyme Mix (a mixture of Int, IHF and Xis). After incubation at 25°C for about 60 minutes, the reaction yields an *amp^r* Expression Clone containing the DNA molecule of interest localized between an *attB1* site and an *attB2* site, and a *kan^r* byproduct molecule, as well as intermediates. The reaction mixture may then be transformed into host cells (*e.g.*, *E. coli*) and clones containing the nucleic acid molecule of interest may

be selected by plating the cells onto ampicillin-containing media and picking amp^r colonies.

Figure 3 is a schematic depiction of the cloning of a nucleic acid molecule from an Entry clone into multiple types of Destination vectors, to produce a variety of Expression Clones. Recombination between a given Entry clone and different types of Destination vectors (not shown), via the LR Reaction depicted in Figure 2, produces multiple different Expression Clones for use in a variety of applications and host cell types.

Figure 4 is a detailed depiction of the production of Entry Clones via a "BP reaction," also referred to herein as an "Entry Reaction" or a "Gateway Reaction." In the example shown in this figure, an amp^r expression vector containing a DNA molecule of interest (*e.g.*, a gene) localized between an *attB1* site and an *attB2* site is reacted with a kan^r Donor vector (*e.g.*, an *attP* vector; here, GATEWAY™ pDONR201 (see Figure 49A-C)) containing a toxic or "death" gene localized between an *attP1* site and an *attP2* site, in the presence of GATEWAY™ BP Clonase™ Enzyme Mix (a mixture of Int and IHF). After incubation at 25°C for about 60 minutes, the reaction yields a kan^r Entry clone containing the DNA molecule of interest localized between an *attL1* site and an *attL2* site, and an amp^r by-product molecule. The Entry clone may then be transformed into host cells (*e.g.*, *E. coli*) and clones containing the Entry clone (and therefore the nucleic acid molecule of interest) may be selected by plating the cells onto kanamycin-containing media and picking kan^r colonies. Although this figure shows an example of use of a kan^r Donor vector, it is also possible to use Donor vectors containing other selection markers, such as the gentamycin resistance or tetracycline resistance markers, as discussed herein.

Figure 5 is a more detailed schematic depiction of the LR ("Destination") reaction (Figure 5A) and the BP ("Entry" or "Gateway") reaction (Figure 5B) of the GATEWAY™ Cloning System, showing the reactants, products and byproducts of each reaction.

Figure 6 shows the sequences of the attB1 and attB2 sites flanking a gene of interest after subcloning into a Destination Vector to create an Expression Clone.

Figure 7 is a schematic depiction of four ways to make Entry Clones using the compositions and methods of the invention: 1. using restriction enzymes and ligase; 2. starting with a cDNA library prepared in an attL Entry Vector; 3. using an Expression Clone from a library prepared in an attB Expression Vector via the BxP reaction; and 4. recombinational cloning of PCR fragments with terminal attB sites, via the BxP reaction. Approaches 3 and 4 rely on recombination with a Donor vector (here, an attP vector such as pDONR201 (see Figure 49A-C), pDONR202 (see Figure 50A-C), pDONR203 (see Figure 51A-C), pDONR204 (see Figure 52A-C), pDONR205 (see Figure 53A-C), or pDONR206 (see Figure 54A-C), for example) that provides an Entry Clone carrying a selection marker such as kan^r, gen^r, tet^r, or the like.

Figure 8 is a schematic depiction of cloning of a PCR product by a BxP (Entry or Gateward) reaction. A PCR product with 25 bp terminal attB sites (plus four Gs) is shown as a substrate for the BxP reaction. Recombination between the attB-PCR product of a gene and a Donor vector (which donates an Entry Vector that carries kan^r) results in an Entry Clone of the PCR product.

Figure 9 is a listing of the nucleotide sequences of the recombination sites designated herein as attB1, attB2, attP1, attP2, attL1, attL2, attR1 and attR2. Sequences are written conventionally, from 5' to 3'.

Figures 10-20: The plasmid backbone for all the Entry Vectors depicted herein is the same, and is shown in Figure 10A for the Entry Vector pENTR1A. For other Entry Vectors shown in Figures 11-20, only the sequences shown in Figure "A" for each figure set (*i.e.*, Figure 11A, Figure 12A, etc.) are different (within the attL1-attL2 cassettes) from those shown in Figure 10 -- the plasmid backbone is identical.

Figure 10 is a schematic depiction of the physical map and cloning sites (Figure 10A), and the nucleotide sequence (Figure 10B), of the Entry Vector pENTR1A.

Figure 11 is a schematic depiction of the cloning sites (Figure 11A) and the nucleotide sequence (Figure 11B) of the Entry Vector pENTR2B.

Figure 12 is a schematic depiction of the cloning sites (Figure 12A) and the nucleotide sequence (Figure 12B) of the Entry Vector pENTR3C.

5 **Figure 13** is a schematic depiction of the cloning sites (Figure 13A) and the nucleotide sequence (Figure 13B) of the Entry Vector pENTR4.

Figure 14 is a schematic depiction of the cloning sites (Figure 14A) and the nucleotide sequence (Figure 14B) of the Entry Vector pENTR5.

10 **Figure 15** is a schematic depiction of the cloning sites (Figure 15A) and the nucleotide sequence (Figure 15B) of the Entry Vector pENTR6.

Figure 16 is a schematic depiction of the cloning sites (Figure 16A) and the nucleotide sequence (Figure 16B) of the Entry Vector pENTR7.

Figure 17 is a schematic depiction of the cloning sites (Figure 17A) and the nucleotide sequence (Figure 17B) of the Entry Vector pENTR8.

15 **Figure 18** is a schematic depiction of the cloning sites (Figure 18A) and the nucleotide sequence (Figure 18B) of the Entry Vector pENTR9.

Figure 19 is a schematic depiction of the cloning sites (Figure 19A) and the nucleotide sequence (Figure 19B) of the Entry Vector pENTR10.

20 **Figure 20** is a schematic depiction of the cloning sites (Figure 20A) and the nucleotide sequence (Figure 20B) of the Entry Vector pENTR11.

Figure 21 is a schematic depiction of the physical map and the Trc expression cassette (Figure 21A) showing the promoter sequences at -35 and at -10 from the initiation codon, and the nucleotide sequence (Figure 21B-D), of Destination Vector pDEST1. This vector may also be referred to as pTrc-DEST1.

25 **Figure 22** is a schematic depiction of the physical map and the His6 expression cassette (Figure 22A) showing the promoter sequences at -35 and at -10 from the initiation codon, and the nucleotide sequence (Figure 22B-D), of Destination Vector pDEST2. This vector may also be referred to as pHis6-DEST2.

30

Figure 23 is a schematic depiction of the physical map and the GST expression cassette (Figure 23A) showing the promoter sequences at -35 and at -10 from the initiation codon, and the nucleotide sequence (Figure 23B-D), of Destination Vector pDEST3. This vector may also be referred to as pGST-DEST3.

Figure 24 is a schematic depiction of the physical map and the His6-Trx expression cassette (Figure 24A) showing the promoter sequences at -35 and at -10 from the initiation codon and a TEV protease cleavage site, and the nucleotide sequence (Figure 24B-D), of Destination Vector pDEST4. This vector may also be referred to as pTrx-DEST4.

Figure 25 is a schematic depiction of the attR1 and attR2 sites (Figure 25A), the physical map (Figure 25B), and the nucleotide sequence (Figure 25C-D), of Destination Vector pDEST5. This vector may also be referred to as pSPORT(+)-DEST5.

Figure 26 is a schematic depiction of the attR1 and attR2 sites (Figure 26A), the physical map (Figure 26B), and the nucleotide sequence (Figure 26C-D), of Destination Vector pDEST6. This vector may also be referred to as pSPORT(-)-DEST6.

Figure 27 is a schematic depiction of the attR1 site, CMV promoter, and the physical map (Figure 27A), and the nucleotide sequence (Figure 27B-C), of Destination Vector pDEST7. This vector may also be referred to as pCMV-DEST7.

Figure 28 is a schematic depiction of the attR1 site, baculovirus polyhedrin promoter, and the physical map (Figure 28A), and the nucleotide sequence (Figure 28B-D), of Destination Vector pDEST8. This vector may also be referred to as pFastBac-DEST8.

Figure 29 is a schematic depiction of the attR1 site, Semliki Forest Virus promoter, and the physical map (Figure 29A), and the nucleotide sequence (Figure 29B-E), of Destination Vector pDEST9. This vector may also be referred to as pSFV-DEST9.

Figure 30 is a schematic depiction of the attR1 site, baculovirus polyhedrin promoter, His6 fusion domain, and the physical map (Figure 30A), and the nucleotide sequence (Figure 30B-D), of Destination Vector pDEST10. This vector may also be referred to as pFastBacHT-DEST10.

Figure 31 is a schematic depiction of the attR1 cassette containing a tetracycline-regulated CMV promoter and the physical map (Figure 31A), and the nucleotide sequence (Figure 31B-D), of Destination Vector pDEST11. This vector may also be referred to as pTet-DEST11.

Figure 32 is a schematic depiction of the attR1 site, the start of the mRNA of the CMV promoter, and the physical map (Figure 32A), and the nucleotide sequence (Figure 32B-D), of Destination Vector pDEST12.2. This vector may also be referred to as pCMVneo-DEST12, as pCMV-DEST12, or as pDEST12.

Figure 33 is a schematic depiction of the attR1 site, the λP_L promoter, and the physical map (Figure 33A), and the nucleotide sequence (Figure 33B-C), of Destination Vector pDEST13. This vector may also be referred to as p λP_L -DEST13.

Figure 34 is a schematic depiction of the attR1 site, the T7 promoter, and the physical map (Figure 34A), and the nucleotide sequence (Figure 34B-D), of Destination Vector pDEST14. This vector may also be referred to as pPT7-DEST14.

Figure 35 is a schematic depiction of the attR1 site, the T7 promoter, and the N-terminal GST fusion sequence, and the physical map (Figure 35A), and the nucleotide sequence (Figure 35B-D), of Destination Vector pDEST15. This vector may also be referred to as pT7 GST-DEST15.

Figure 36 is a schematic depiction of the attR1 site, the T7 promoter, and the N-terminal thioredoxin fusion sequence, and the physical map (Figure 36A), and the nucleotide sequence (Figure 36B-D), of Destination Vector pDEST16. This vector may also be referred to as pT7 Trx-DEST16.

Figure 37 is a schematic depiction of the attR1 site, the T7 promoter, and the N-terminal His6 fusion sequence, and the physical map (Figure 37A), and the

nucleotide sequence (Figure 37B-D), of Destination Vector pDEST17. This vector may also be referred to as pT7 His-DEST17.

Figure 38 is a schematic depiction of the attR1 site and the p10 baculovirus promoter, and the physical map (Figure 38A), and the nucleotide sequence (Figure 38B-D), of Destination Vector pDEST18. This vector may also be referred to as pFBp10-DEST18.

Figure 39 is a schematic depiction of the attR1 site, and the 39k baculovirus promoter, and the physical map (Figure 39A), and the nucleotide sequence (Figure 39B-D), of Destination Vector pDEST19. This vector may also be referred to as pFB39k-DEST19.

Figure 40 is a schematic depiction of the attR1 site, the *polh* baculovirus promoter, and the N-terminal GST fusion sequence, and the physical map (Figure 40A), and the nucleotide sequence (Figure 40B-D), of Destination Vector pDEST20. This vector may also be referred to as pFB GST-DEST20.

Figure 41 is a schematic depiction of a 2-hybrid vector with a DNA-binding domain, the attR1 site, and the ADH promoter, and the physical map (Figure 41A), and the nucleotide sequence (Figure 41B-E), of Destination Vector pDEST21. This vector may also be referred to as pDB Leu-DEST21.

Figure 42 is a schematic depiction of a 2-hybrid vector with an activation domain, the attR1 site, and the ADH promoter, and the physical map (Figure 42A), and the nucleotide sequence (Figure 42B-D), of Destination Vector pDEST22. This vector may also be referred to as pPC86-DEST22.

Figure 43 is a schematic depiction of the attR1 and attR2 sites, the T7 promoter, and the C-terminal His6 fusion sequence, and the physical map (Figure 43A), and the nucleotide sequence (Figure 43B-D), of Destination Vector pDEST23. This vector may also be referred to as pC-term-His6-DEST23.

Figure 44 is a schematic depiction of the attR1 and attR2 sites, the T7 promoter, and the C-terminal GST fusion sequence, and the physical map (Figure 44A), and the nucleotide sequence (Figure 44B-D), of Destination Vector pDEST24. This vector may also be referred to as pC-term-GST-DEST24.

Figure 45 is a schematic depiction of the attR1 and attR2 sites, the T7 promoter, and the C-terminal thioredoxin fusion sequence, and the physical map (Figure 45A), and the nucleotide sequence (Figure 45B-D), of Destination Vector pDEST25. This vector may also be referred to as pC-term-Trx-DEST25.

Figure 46 is a schematic depiction of the attR1 site, the CMV promoter, and an N-terminal His6 fusion sequence, and the physical map (Figure 46A), and the nucleotide sequence (Figure 46B-D), of Destination Vector pDEST26. This vector may also be referred to as pCMV-SPneo-His-DEST26.

Figure 47 is a schematic depiction of the attR1 site, the CMV promoter, and an N-terminal GST fusion sequence, and the physical map (Figure 47A), and the nucleotide sequence (Figure 47B-D), of Destination Vector pDEST27. This vector may also be referred to as pCMV-Spneo-GST-DEST27.

Figure 48 is a depiction of the physical map (Figure 48A), the cloning sites (Figure 48B), and the nucleotide sequence (Figure 48C-D), for the attB cloning vector plasmid pEXP501. This vector may also be referred to equivalently herein as pCMV•SPORT6, pCMVSPORT6, and pCMVSpport6.

Figure 49 is a depiction of the physical map (Figure 49A), and the nucleotide sequence (Figure 49B-C), for the Donor plasmid pDONR201 which donates a kanamycin-resistant vector in the BP Reaction. This vector may also be referred to as pAttPkanr Donor Plasmid, or as pAttPkan Donor Plasmid

Figure 50 is a depiction of the physical map (Figure 50A), and the nucleotide sequence (Figure 50B-C), for the Donor plasmid pDONR202 which donates a kanamycin-resistant vector in the BP Reaction.

Figure 51 is a depiction of the physical map (Figure 51A), and the nucleotide sequence (Figure 51B-C), for the Donor plasmid pDONR203 which donates a kanamycin-resistant vector in the BP Reaction.

Figure 52 is a depiction of the physical map (Figure 52A), and the nucleotide sequence (Figure 52B-C), for the Donor plasmid pDONR204 which donates a kanamycin-resistant vector in the BP Reaction.

Figure 53 is a depiction of the physical map (Figure 53A), and the nucleotide sequence (Figure 53B-C), for the Donor plasmid pDONR205 which donates a tetracycline-resistant vector in the BP Reaction.

Figure 54 is a depiction of the physical map (Figure 54A), and the nucleotide sequence (Figure 54B-C), for the Donor plasmid pDONR206 which donates a gentamycin-resistant vector in the BP Reaction. This vector may also be referred to as pENTR22 attP Donor Plasmid, pAttPGenr Donor Plasmid, or pAttPgnt Donor Plasmid.

Figure 55 depicts the attB1 site, and the physical map, of an Entry Clone (pENTR7) of CAT subcloned into the Destination Vector pDEST2 (Figure 22).

Figure 56 depicts the DNA components of Reaction B of the one-tube BxP reaction described in Example 16, pEZX7102 and attB-tet-PCR.

Figure 57 is a physical map of the desired product of Reaction B of the one-tube BxP reaction described in Example 16, tetx7102.

Figure 58 is a physical map of the Destination Vector pEZX8402.

Figure 59 is a physical map of the expected tet^r subclone product, tetx8402, resulting from the LxR Reaction with tetx7102 (Figure 57) plus pEZX8402 (Figure 58).

Figure 60 is a schematic depiction of the bacteriophage lambda recombination pathways in *E. coli*.

Figure 61 is a schematic depiction of the DNA molecules participating in the LR Reaction. Two different co-integrates form during the LR Reaction (only one of which is shown here), depending on whether attL1 and attR1 or attL2 and attR2 are first to recombine. In one aspect, the invention provides directional cloning of a nucleic acid molecule of interest, since the recombination sites react with specificity (attL1 reacts with attR1; attL2 with attR2; attB1 with attP1; and attB2 with attP2). Thus, positioning of the sites allows construction of desired vectors having recombined fragments in the desired orientation.

Figure 62 is a depiction of native and fusion protein expression using the recombinational cloning methods and compositions of the invention. In the upper figure depicting native protein expression, all of the translational start signals are

included between the attB1 and attB2 sites; therefore, these signals must be present in the starting Entry Clone. The lower figure depicts fusion protein expression (here showing expression with both N-terminal and C-terminal fusion tags so that ribosomes read through attB1 and attB2 to create the fusion protein). Unlike native protein expression vectors, N-terminal fusion vectors have their translational start signals upstream of the attB1 site.

Figure 63 is a schematic depiction of three GATEWAY™ Cloning System cassettes. Three blunt-ended cassettes are depicted which convert standard expression vectors to Destination Vectors. Each of the depicted cassettes provides amino-terminal fusions in one of three possible reading frames, and each has a distinctive restriction cleavage site as shown.

Figure 64 shows the physical maps of plasmids containing three attR reading frame cassettes, pEZC15101 (reading frame A; Figure 64A), pEZC15102 (reading frame B; Figure 64B), and pEZC15103 (reading frame C; Figure 64C).

Figure 65 depicts the attB primers used for amplifying the tet^r and amp^r genes from pBR322 by the cloning methods of the invention.

Figure 66 is a table listing the results of recombinational cloning of the tet^r and amp^r PCR products made using the primers shown in Figure 65.

Figure 67 is a graph showing the effect of the number of guanines (G's) contained on the 5' end of the PCR primers on the cloning efficiency of PCR products. It is noted, however, that other nucleotides besides guanine (including A, T, C, U or combinations thereof) may be used as 5' extensions on the PCR primers to enhance cloning efficiency of PCR products.

Figure 68 is a graph showing a titration of various amounts of attP and attB reactants in the BxP reaction, and the effects on cloning efficiency of PCR products.

Figure 69 is a series of graphs showing the effects of various weights (Figure 69A) or moles (Figure 69B) of a 256 bp PCR product on formation of colonies, and on efficiency of cloning of the 256 bp PCR product into a Donor Vector (Figure 69C).

Figure 70 is a series of graphs showing the effects of various weights (Figure 70A) or moles (Figure 70B) of a 1 kb PCR product on formation of colonies, and on efficiency of cloning of the 1 kb PCR product into a Donor Vector (Figure 70C).

Figure 71 is a series of graphs showing the effects of various weights (Figure 71A) or moles (Figure 71B) of a 1.4 kb PCR product on formation of colonies, and on efficiency of cloning of the 1.4 kb PCR product into a Donor Vector (Figure 71C).

Figure 72 is a series of graphs showing the effects of various weights (Figure 72A) or moles (Figure 72B) of a 3.4 kb PCR product on formation of colonies, and on efficiency of cloning of the 3.4 kb PCR product into a Donor Vector (Figure 72C).

Figure 73 is a series of graphs showing the effects of various weights (Figure 73A) or moles (Figure 73B) of a 4.6 kb PCR product on formation of colonies, and on efficiency of cloning of the 4.6 kb PCR product into a Donor Vector (Figure 73C).

Figure 74 is photograph of an ethidium bromide-stained gel of a titration of a 6.9 kb PCR product in a BxP reaction.

Figure 75 is a graph showing the effects of various amounts of a 10.1 kb PCR product on formation of colonies upon cloning of the 10.1 kb PCR product into a Donor Vector.

Figure 76 is photograph of an ethidium bromide-stained gel of a titration of a 10.1 kb PCR product in a BxP reaction.

Figure 77 is a table summarizing the results of the PCR product cloning efficiency experiments depicted in Figures 69-74, for PCR fragments ranging in size from 0.256 kb to 6.9 kb.

Figure 78 is a depiction of the sequences at the ends of attR Cassettes. Sequences contributed by the Cm^r -ccdB cassette are shown, including the outer ends of the flanking attR sites (boxed). The staggered cleavage sites for Int are indicated in the boxed regions. Following recombination with an Entry Clone, only the outer sequences in attR sites contribute to the resulting attB sites in the

Expression Clone. The underlined sequences at both ends dictate the different reading frames (reading frames A, B, or C, with two alternative reading frame C cassettes depicted) for fusion proteins.

5 **Figure 79** is a depiction of several different attR cassettes (in reading frames A, B, or C) which may provide fusion codons at the amino-terminus of the encoded protein.

Figure 80 illustrates the single-cutting restriction sites in an attR reading frame A cassette of the invention.

10 **Figure 81** illustrates the single-cutting restriction sites in an attR reading frame B cassette of the invention.

Figure 82 illustrates the single-cutting restriction sites in two alternative attR reading frame C cassettes of the invention (Figures 82A and 82B) depicted in Figure 78.

15 **Figure 83** shows the physical map (Figure 83A), and the nucleotide sequence (Figure 83B-C), for an attR reading frame C parent plasmid prfC Parent III, which contains an attR reading frame C cassette of the invention (alternative A in Figures 78 and 82).

Figure 84 is a physical map of plasmid pEZC1301.

Figure 85 is a physical map of plasmid pEZC1313.

20 **Figure 86** is a physical map of plasmid pEZ14032.

Figure 87 is a physical map of plasmid pMAB58.

Figure 88 is a physical map of plasmid pMAB62.

Figure 89 is a depiction of a synthesis reaction using two pairs of homologous primers of the invention.

25 **Figure 90** is a schematic depiction of the physical map (Figure 90A), and the nucleotide sequence (Figure 90B-D), of Destination Vector pDEST28.

Figure 91 is a schematic depiction of the physical map (Figure 91A), and the nucleotide sequence (Figure 91B-D), of Destination Vector pDEST29.

30 **Figure 92** is a schematic depiction of the physical map (Figure 92A), and the nucleotide sequence (Figure 92B-D), of Destination Vector pDEST30.

Figure 93 is a schematic depiction of the physical map (Figure 93A), and the nucleotide sequence (Figure 93B-D), of Destination Vector pDEST31.

Figure 94 is a schematic depiction of the physical map (Figure 94A), and the nucleotide sequence (Figure 94B-E), of Destination Vector pDEST32.

5 **Figure 95** is a schematic depiction of the physical map (Figure 95A), and the nucleotide sequence (Figure 95B-D), of Destination Vector pDEST33.

Figure 96 is a schematic depiction of the physical map (Figure 96A), and the nucleotide sequence (Figure 96B-D), of Destination Vector pDEST34.

10 **Figure 97** is a depiction of the physical map (Figure 97A), and the nucleotide sequence (Figure 97B-C), for the Donor plasmid pDONR207 which donates a gentamycin-resistant vector in the BP Reaction.

Figure 98 is a schematic depiction of the physical map (Figure 98A), and the nucleotide sequence (Figure 98B-D), of the 2-hybrid vector pMAB85.

15 **Figure 99** is a schematic depiction of the physical map (Figure 99A), and the nucleotide sequence (Figure 99B-D), of the 2-hybrid vector pMAB86.

DETAILED DESCRIPTION OF THE INVENTION

20 *Definitions*

In the description that follows, a number of terms used in recombinant DNA technology are utilized extensively. In order to provide a clear and consistent understanding of the specification and claims, including the scope to be given such terms, the following definitions are provided.

25 **Byproduct:** is a daughter molecule (a new clone produced after the second recombination event during the recombinational cloning process) lacking the segment which is desired to be cloned or subcloned.

30 **Cointegrate:** is at least one recombination intermediate nucleic acid molecule of the present invention that contains both parental (starting) molecules. It will usually be linear. In some embodiments it can be circular. RNA and polypeptides may be expressed from cointegrates using an appropriate host cell strain, for example *E. coli* DB3.1 (particularly *E. coli* LIBRARY EFFICIENCY®).

DB3.1™ Competent Cells), and selecting for both selection markers found on the cointegrate molecule.

Host: is any prokaryotic or eukaryotic organism that can be a recipient of the recombinational cloning Product, vector, or nucleic acid molecule of the invention. A "host," as the term is used herein, includes prokaryotic or eukaryotic organisms that can be genetically engineered. For examples of such hosts, *see* Maniatis *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (1982).

Insert or Inserts: include the desired nucleic acid segment or a population of nucleic acid segments (segment *A* of Figure 1) which may be manipulated by the methods of the present invention. Thus, the terms Insert(s) are meant to include a particular nucleic acid (preferably DNA) segment or a population of segments. Such Insert(s) can comprise one or more nucleic acid molecules.

Insert Donor: is one of the two parental nucleic acid molecules (e.g. RNA or DNA) of the present invention which carries the Insert. The Insert Donor molecule comprises the Insert flanked on both sides with recombination sites. The Insert Donor can be linear or circular. In one embodiment of the invention, the Insert Donor is a circular DNA molecule and further comprises a cloning vector sequence outside of the recombination signals (see Figure 1). When a population of Inserts or population of nucleic acid segments are used to make the Insert Donor, a population of Insert Donors results and may be used in accordance with the invention. Examples of such Insert Donor molecules are GATEWAY™ Entry Vectors, which include but are not limited to those Entry Vectors depicted in Figures 10-20, as well as other vectors comprising a gene of interest flanked by one or more *attL* sites (e.g., *attL1*, *attL2*, etc.), or by one or more *attB* sites (e.g., *attB1*, *attB2*, etc.) for the production of library clones.

Product: is one of the desired daughter molecules comprising the *A* and *D* sequences which is produced after the second recombination event during the recombinational cloning process (see Figure 1). The Product contains the nucleic acid which was to be cloned or subcloned. In accordance with the invention, when a population of Insert Donors are used, the resulting population of Product

molecules will contain all or a portion of the population of Inserts of the Insert Donors and preferably will contain a representative population of the original molecules of the Insert Donors.

Promoter: is a DNA sequence generally described as the 5'-region of a gene, located proximal to the start codon. The transcription of an adjacent DNA segment is initiated at the promoter region. A repressible promoter's rate of transcription decreases in response to a repressing agent. An inducible promoter's rate of transcription increases in response to an inducing agent. A constitutive promoter's rate of transcription is not specifically regulated, though it can vary under the influence of general metabolic conditions.

Recognition sequence: Recognition sequences are particular sequences which a protein, chemical compound, DNA, or RNA molecule (*e.g.*, restriction endonuclease, a modification methylase, or a recombinase) recognizes and binds. In the present invention, a recognition sequence will usually refer to a recombination site. For example, the recognition sequence for Cre recombinase is *loxP* which is a 34 base pair sequence comprised of two 13 base pair inverted repeats (serving as the recombinase binding sites) flanking an 8 base pair core sequence. See Figure 1 of Sauer, B., *Current Opinion in Biotechnology* 5:521-527 (1994). Other examples of recognition sequences are the *attB*, *attP*, *attL*, and *attR* sequences which are recognized by the recombinase enzyme λ Integrase. *attB* is an approximately 25 base pair sequence containing two 9 base pair core-type Int binding sites and a 7 base pair overlap region. *attP* is an approximately 240 base pair sequence containing core-type Int binding sites and arm-type Int binding sites as well as sites for auxiliary proteins integration host factor (IHF), FIS and excisionase (Xis). See Landy, *Current Opinion in Biotechnology* 3:699-707 (1993). Such sites may also be engineered according to the present invention to enhance production of products in the methods of the invention. When such engineered sites lack the P1 or H1 domains to make the recombination reactions irreversible (*e.g.*, *attR* or *attP*), such sites may be designated *attR'* or *attP'* to show that the domains of these sites have been modified in some way.

Recombination proteins: include excisive or integrative proteins, enzymes, co-factors or associated proteins that are involved in recombination reactions involving one or more recombination sites, which may be wild-type proteins (See Landy, *Current Opinion in Biotechnology* 3:699-707 (1993)), or mutants, derivatives (e.g., fusion proteins containing the recombination protein sequences or fragments thereof), fragments, and variants thereof.

Recombination site: is a recognition sequence on a DNA molecule participating in an integration/recombination reaction by the recombinational cloning methods of the invention. Recombination sites are discrete sections or segments of DNA on the participating nucleic acid molecules that are recognized and bound by a site-specific recombination protein during the initial stages of integration or recombination. For example, the recombination site for Cre recombinase is *loxP* which is a 34 base pair sequence comprised of two 13 base pair inverted repeats (serving as the recombinase binding sites) flanking an 8 base pair core sequence. See Figure 1 of Sauer, B., *Curr. Opin. Biotech.* 5:521-527 (1994). Other examples of recognition sequences include the *attB*, *attP*, *attL*, and *attR* sequences described herein, and mutants, fragments, variants and derivatives thereof, which are recognized by the recombination protein λ Int and by the auxiliary proteins integration host factor (IHF), FIS and excisionase (Xis). See Landy, *Curr. Opin. Biotech.* 3:699-707 (1993).

Recombinational Cloning: is a method described herein, whereby segments of nucleic acid molecules or populations of such molecules are exchanged, inserted, replaced, substituted or modified, *in vitro* or *in vivo*. By “*in vitro*” and “*in vivo*” herein is meant recombinational cloning that is carried out outside of host cells (e.g., in cell-free systems) or inside of host cells (e.g., using recombination proteins expressed by host cells), respectively.

Repression cassette: is a nucleic acid segment that contains a repressor or a Selectable marker present in the subcloning vector.

Selectable marker: is a DNA segment that allows one to select for or against a molecule (e.g., a replicon) or a cell that contains it, often under particular conditions. These markers can encode an activity, such as, but not limited to,

production of RNA, peptide, or protein, or can provide a binding site for RNA, peptides, proteins, inorganic and organic compounds or compositions and the like. Examples of Selectable markers include but are not limited to: (1) DNA segments that encode products which provide resistance against otherwise toxic compounds (e.g., antibiotics); (2) DNA segments that encode products which are otherwise lacking in the recipient cell (e.g., tRNA genes, auxotrophic markers); (3) DNA segments that encode products which suppress the activity of a gene product; (4) DNA segments that encode products which can be readily identified (e.g., phenotypic markers such as β -galactosidase, green fluorescent protein (GFP), and cell surface proteins); (5) DNA segments that bind products which are otherwise detrimental to cell survival and/or function; (6) DNA segments that otherwise inhibit the activity of any of the DNA segments described in Nos. 1-5 above (e.g., antisense oligonucleotides); (7) DNA segments that bind products that modify a substrate (e.g. restriction endonucleases); (8) DNA segments that can be used to isolate or identify a desired molecule (e.g. specific protein binding sites); (9) DNA segments that encode a specific nucleotide sequence which can be otherwise non-functional (e.g., for PCR amplification of subpopulations of molecules); (10) DNA segments, which when absent, directly or indirectly confer resistance or sensitivity to particular compounds; (11) DNA segments that encode products which are toxic in recipient cells; (12) DNA segments that inhibit replication, partition or heritability of nucleic acid molecules that contain them; and/or (13) DNA segments that encode conditional replication functions, e.g., replication in certain hosts or host cell strains or under certain environmental conditions (e.g., temperature, nutritional conditions, etc.).

Selection scheme: is any method which allows selection, enrichment, or identification of a desired Product or Product(s) from a mixture containing an Entry Clone or Vector, a Destination Vector, a Donor Vector, an Expression Clone or Vector, any intermediates (e.g. a Cointegrate or a replicon), and/or Byproducts. The selection schemes of one preferred embodiment have at least two components that are either linked or unlinked during recombinational cloning. One component is a Selectable marker. The other component controls the expression *in vitro* or *in vivo* of the Selectable marker, or survival of the cell (or

the nucleic acid molecule, *e.g.*, a replicon) harboring the plasmid carrying the Selectable marker. Generally, this controlling element will be a repressor or inducer of the Selectable marker, but other means for controlling expression or activity of the Selectable marker can be used. Whether a repressor or activator is used will depend on whether the marker is for a positive or negative selection, and the exact arrangement of the various DNA segments, as will be readily apparent to those skilled in the art. A preferred requirement is that the selection scheme results in selection of or enrichment for only one or more desired Products. As defined herein, selecting for a DNA molecule includes (a) selecting or enriching for the presence of the desired DNA molecule, and (b) selecting or enriching against the presence of DNA molecules that are not the desired DNA molecule.

In one embodiment, the selection schemes (which can be carried out in reverse) will take one of three forms, which will be discussed in terms of Figure 1. The first, exemplified herein with a Selectable marker and a repressor therefore, selects for molecules having segment *D* and lacking segment *C*. The second selects against molecules having segment *C* and for molecules having segment *D*. Possible embodiments of the second form would have a DNA segment carrying a gene toxic to cells into which the *in vitro* reaction products are to be introduced. A toxic gene can be a DNA that is expressed as a toxic gene product (a toxic protein or RNA), or can be toxic in and of itself. (In the latter case, the toxic gene is understood to carry its classical definition of "heritable trait".)

Examples of such toxic gene products are well known in the art, and include, but are not limited to, restriction endonucleases (*e.g.*, *DpnI*), apoptosis-related genes (*e.g.* ASK1 or members of the *bcl-2/ced-9* family), retroviral genes including those of the human immunodeficiency virus (HIV), defensins such as NP-1, inverted repeats or paired palindromic DNA sequences, bacteriophage lytic genes such as those from Φ X174 or bacteriophage T4; antibiotic sensitivity genes such as *rpsL*, antimicrobial sensitivity genes such as *pheS*, plasmid killer genes, eukaryotic transcriptional vector genes that produce a gene product toxic to bacteria, such as GATA-1, and genes that kill hosts in the absence of a suppressing function, *e.g.*, *kicB*, *ccdB*, Φ X174 *E* (Liu, Q. *et al.*, *Curr. Biol.*

8:1300-1309 (1998)), and other genes that negatively affect replicon stability and/or replication. A toxic gene can alternatively be selectable *in vitro*, e.g., a restriction site.

Many genes coding for restriction endonucleases operably linked to inducible promoters are known, and may be used in the present invention. See, e.g. U.S. Patent Nos. 4,960,707 (*DpnI* and *DpnII*); 5,000,333, 5,082,784 and 5,192,675 (*KpnI*); 5,147,800 (*NgoAIII* and *NgoAI*); 5,179,015 (*FspI* and *HaeIII*); 5,200,333 (*HaeII* and *TaqI*); 5,248,605 (*HpaII*); 5,312,746 (*ClaI*); 5,231,021 and 5,304,480 (*XhoI* and *XhoII*); 5,334,526 (*AluI*); 5,470,740 (*NsiI*); 5,534,428 (*SstI/SacI*); 5,202,248 (*NcoI*); 5,139,942 (*NdeI*); and 5,098,839 (*PacI*). See also Wilson, G.G., *Nucl. Acids Res.* 19:2539-2566 (1991); and Lunnen, K.D., *et al.*, *Gene* 74:25-32 (1988).

In the second form, segment *D* carries a Selectable marker. The toxic gene would eliminate transformants harboring the Vector Donor, Cointegrate, and Byproduct molecules, while the Selectable marker can be used to select for cells containing the Product and against cells harboring only the Insert Donor.

The third form selects for cells that have both segments *A* and *D* in *cis* on the same molecule, but not for cells that have both segments in *trans* on different molecules. This could be embodied by a Selectable marker that is split into two inactive fragments, one each on segments *A* and *D*.

The fragments are so arranged relative to the recombination sites that when the segments are brought together by the recombination event, they reconstitute a functional Selectable marker. For example, the recombinational event can link a promoter with a structural nucleic acid molecule (e.g., a gene), can link two fragments of a structural nucleic acid molecule, or can link nucleic acid molecules that encode a heterodimeric gene product needed for survival, or can link portions of a replicon.

Site-specific recombinase: is a type of recombinase which typically has at least the following four activities (or combinations thereof): (1) recognition of one or two specific nucleic acid sequences; (2) cleavage of said sequence or sequences; (3) topoisomerase activity involved in strand exchange; and (4) ligase

activity to reseal the cleaved strands of nucleic acid. See Sauer, B., *Current Opinions in Biotechnology* 5:521-527 (1994). Conservative site-specific recombination is distinguished from homologous recombination and transposition by a high degree of sequence specificity for both partners. The strand exchange mechanism involves the cleavage and rejoining of specific DNA sequences in the absence of DNA synthesis (Landy, A. (1989) *Ann. Rev. Biochem.* 58:913-949).

Subcloning vector: is a cloning vector comprising a circular or linear nucleic acid molecule which includes preferably an appropriate replicon. In the present invention, the subcloning vector (segment *D* in Figure 1) can also contain functional and/or regulatory elements that are desired to be incorporated into the final product to act upon or with the cloned DNA Insert (segment *A* in Figure 1). The subcloning vector can also contain a Selectable marker (preferably DNA).

Vector: is a nucleic acid molecule (preferably DNA) that provides a useful biological or biochemical property to an Insert. Examples include plasmids, phages, autonomously replicating sequences (ARS), centromeres, and other sequences which are able to replicate or be replicated *in vitro* or in a host cell, or to convey a desired nucleic acid segment to a desired location within a host cell. A Vector can have one or more restriction endonuclease recognition sites at which the sequences can be cut in a determinable fashion without loss of an essential biological function of the vector, and into which a nucleic acid fragment can be spliced in order to bring about its replication and cloning. Vectors can further provide primer sites, *e.g.*, for PCR, transcriptional and/or translational initiation and/or regulation sites, recombinational signals, replicons, Selectable markers, *etc.* Clearly, methods of inserting a desired nucleic acid fragment which do not require the use of homologous recombination, transpositions or restriction enzymes (such as, but not limited to, UDG cloning of PCR fragments (U.S. Patent No. 5,334,575, entirely incorporated herein by reference), T:A cloning, and the like) can also be applied to clone a fragment into a cloning vector to be used according to the present invention. The cloning vector can further contain one or more selectable markers suitable for use in the identification of cells transformed with the cloning vector.

Vector Donor: is one of the two parental nucleic acid molecules (e.g. RNA or DNA) of the present invention which carries the DNA segments comprising the DNA vector which is to become part of the desired Product. The Vector Donor comprises a subcloning vector *D* (or it can be called the cloning vector if the Insert Donor does not already contain a cloning vector (e.g., for PCR fragments containing *attB* sites; see below)) and a segment *C* flanked by recombination sites (see Figure 1). Segments *C* and/or *D* can contain elements that contribute to selection for the desired Product daughter molecule, as described above for selection schemes. The recombination signals can be the same or different, and can be acted upon by the same or different recombinases. In addition, the Vector Donor can be linear or circular. Examples of such Vector Donor molecules include GATEWAY™ Destination Vectors, which include but are not limited to those Destination Vectors depicted in Figures 21-47 and 90-96.

Primer: refers to a single stranded or double stranded oligonucleotide that is extended by covalent bonding of nucleotide monomers during amplification or polymerization of a nucleic acid molecule (e.g. a DNA molecule). In a preferred aspect, a primer comprises one or more recombination sites or portions of such recombination sites. Portions of recombination sites comprise at least 2 bases (or basepairs, abbreviated herein as "bp"), at least 5-200 bases, at least 10-100 bases, at least 15-75 bases, at least 15-50 bases, at least 15-25 bases, or at least 16-25 bases, of the recombination sites of interest, as described in further detail below and in the Examples. When using portions of recombination sites, the missing portion of the recombination site may be provided as a template by the newly synthesized nucleic acid molecule. Such recombination sites may be located within and/or at one or both termini of the primer. Preferably, additional sequences are added to the primer adjacent to the recombination site(s) to enhance or improve recombination and/or to stabilize the recombination site during recombination. Such stabilization sequences may be any sequences (preferably G/C rich sequences) of any length. Preferably, such sequences range in size from 1 to about 1000 bases, 1 to about 500 bases, and 1 to about 100 bases, 1 to about 60 bases, 1 to about 25, 1 to about 10, 2 to about 10 and preferably about 4 bases.

Preferably, such sequences are greater than 1 base in length and preferably greater than 2 bases in length.

Template: refers to double stranded or single stranded nucleic acid molecules which are to be amplified, synthesized or sequenced. In the case of double stranded molecules, denaturation of its strands to form a first and a second strand is preferably performed before these molecules will be amplified, synthesized or sequenced, or the double stranded molecule may be used directly as a template. For single stranded templates, a primer complementary to a portion of the template is hybridized under appropriate conditions and one or more polypeptides having polymerase activity (e.g. DNA polymerases and/or reverse transcriptases) may then synthesize a nucleic acid molecule complementary to all or a portion of said template. Alternatively, for double stranded templates, one or more promoters may be used in combination with one or more polymerases to make nucleic acid molecules complementary to all or a portion of the template. The newly synthesized molecules, according to the invention, may be equal or shorter in length than the original template. Additionally, a population of nucleic acid templates may be used during synthesis or amplification to produce a population of nucleic acid molecules typically representative of the original template population.

Adapter: is an oligonucleotide or nucleic acid fragment or segment (preferably DNA) which comprises one or more recombination sites (or portions of such recombination sites) which in accordance with the invention can be added to a circular or linear Insert Donor molecule as well as other nucleic acid molecules described herein. When using portions of recombination sites, the missing portion may be provided by the Insert Donor molecule. Such adapters may be added at any location within a circular or linear molecule, although the adapters are preferably added at or near one or both termini of a linear molecule. Preferably, adapters are positioned to be located on both sides (flanking) a particular nucleic acid molecule of interest. In accordance with the invention, adapters may be added to nucleic acid molecules of interest by standard recombinant techniques (e.g. restriction digest and ligation). For example, adapters may be added to a circular molecule by first digesting the molecule with

an appropriate restriction enzyme, adding the adapter at the cleavage site and reforming the circular molecule which contains the adapter(s) at the site of cleavage. In other aspects, adapters may be added by homologous recombination, by integration of RNA molecules, and the like. Alternatively, adapters may be ligated directly to one or more and preferably both termini of a linear molecule thereby resulting in linear molecule(s) having adapters at one or both termini. In one aspect of the invention, adapters may be added to a population of linear molecules, (e.g. a cDNA library or genomic DNA which has been cleaved or digested) to form a population of linear molecules containing adapters at one and preferably both termini of all or substantial portion of said population.

Adapter-Primer: is primer molecule which comprises one or more recombination sites (or portions of such recombination sites) which in accordance with the invention can be added to a circular or linear nucleic acid molecule described herein. When using portions of recombination sites, the missing portion may be provided by a nucleic acid molecule (e.g., an adapter) of the invention. Such adapter-primers may be added at any location within a circular or linear molecule, although the adapter-primers are preferably added at or near one or both termini of a linear molecule. Examples of such adapter-primers and the use thereof in accordance with the methods of the invention are shown in Example 25 herein. Such adapter-primers may be used to add one or more recombination sites or portions thereof to circular or linear nucleic acid molecules in a variety of contexts and by a variety of techniques, including but not limited to amplification (e.g., PCR), ligation (e.g., enzymatic or chemical/synthetic ligation), recombination (e.g., homologous or non-homologous (illegitimate) recombination) and the like.

Library: refers to a collection of nucleic acid molecules (circular or linear). In one embodiment, a library may comprise a plurality (i.e., two or more) of DNA molecules, which may or may not be from a common source organism, organ, tissue, or cell. In another embodiment, a library is representative of all or a portion or a significant portion of the DNA content of an organism (a "genomic" library), or a set of nucleic acid molecules representative of all or a portion or a significant portion of the expressed nucleic acid molecules (a cDNA library) in a

cell, tissue, organ or organism. A library may also comprise random sequences made by *de novo* synthesis, mutagenesis of one or more sequences and the like. Such libraries may or may not be contained in one or more vectors.

Amplification: refers to any *in vitro* method for increasing a number of copies of a nucleotide sequence with the use of a polymerase. Nucleic acid amplification results in the incorporation of nucleotides into a DNA and/or RNA molecule or primer thereby forming a new molecule complementary to a template. The formed nucleic acid molecule and its template can be used as templates to synthesize additional nucleic acid molecules. As used herein, one amplification reaction may consist of many rounds of replication. DNA amplification reactions include, for example, polymerase chain reaction (PCR). One PCR reaction may consist of 5-100 "cycles" of denaturation and synthesis of a DNA molecule.

Oligonucleotide: refers to a synthetic or natural molecule comprising a covalently linked sequence of nucleotides which are joined by a phosphodiester bond between the 3' position of the deoxyribose or ribose of one nucleotide and the 5' position of the deoxyribose or ribose of the adjacent nucleotide. This term may be used interchangeably herein with the terms "nucleic acid molecule" and "polynucleotide," without any of these terms necessarily indicating any particular length of the nucleic acid molecule to which the term specifically refers.

Nucleotide: refers to a base-sugar-phosphate combination. Nucleotides are monomeric units of a nucleic acid molecule (DNA and RNA). The term nucleotide includes ribonucleoside triphosphates ATP, UTP, CTG, GTP and deoxyribonucleoside triphosphates such as dATP, dCTP, dITP, dUTP, dGTP, dTTP, or derivatives thereof. Such derivatives include, for example, [α S]dATP, 7-deaza-dGTP and 7-deaza-dATP. The term nucleotide as used herein also refers to dideoxyribonucleoside triphosphates (ddNTPs) and their derivatives. Illustrated examples of dideoxyribonucleoside triphosphates include, but are not limited to, ddATP, ddCTP, ddGTP, ddITP, and ddTTP. According to the present invention, a "nucleotide" may be unlabeled or detectably labeled by well known techniques. Detectable labels include, for example, radioactive isotopes, fluorescent labels, chemiluminescent labels, bioluminescent labels and enzyme labels.

Hybridization: The terms “hybridization” and “hybridizing” refers to base pairing of two complementary single-stranded nucleic acid molecules (RNA and/or DNA) to give a double stranded molecule. As used herein, two nucleic acid molecules may be hybridized, although the base pairing is not completely complementary. Accordingly, mismatched bases do not prevent hybridization of two nucleic acid molecules provided that appropriate conditions, well known in the art, are used. In some aspects, hybridization is said to be under “stringent conditions.” By “stringent conditions” as used herein is meant overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (150 mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt’s solution, 10% dextran sulfate, and 20 g/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C.

Other terms used in the fields of recombinant DNA technology and molecular and cell biology as used herein will be generally understood by one of ordinary skill in the applicable arts.

Overview

Two reactions constitute the recombinational cloning system of the present invention, referred to herein as the “GATEWAY™ Cloning System,” as depicted generally in Figure 1. The first of these reactions, the **LR Reaction** (Figure 2), which may also be referred to interchangeably herein as the **Destination Reaction**, is the main pathway of this system. The LR Reaction is a recombination reaction between an Entry vector or clone and a Destination Vector, mediated by a cocktail of recombination proteins such as the GATEWAY™ LR Clonase™ Enzyme Mix described herein. This reaction transfers nucleic acid molecules of interest (which may be genes, cDNAs, cDNA libraries, or fragments thereof) from the Entry Clone to an Expression Vector, to create an Expression Clone.

The sites labeled L, R, B, and P are respectively the attL, attR, attB, and attP recombination sites for the bacteriophage λ recombination proteins that constitute the Clonase cocktail (referred to herein variously as “Clonase” or

“GATEWAY™ LR Clonase™ Enzyme Mix” (for recombination protein mixtures mediating attL x attR recombination reactions, as described herein) or “GATEWAY™ BP Clonase™ Enzyme Mix” (for recombination protein mixtures mediating attB x attP recombination reactions, as described herein)). The Recombinational Cloning reactions are equivalent to concerted, highly specific, cutting and ligation reactions. Viewed in this way, the recombination proteins cut to the left and right of the nucleic acid molecule of interest in the Entry Clone and ligate it into the Destination vector, creating a new Expression Clone.

The nucleic acid molecule of interest in an Expression Clone is flanked by the small attB1 and attB2 sites. The orientation and reading frame of the nucleic acid molecule of interest are maintained throughout the subcloning, because attL1 reacts only with attR1, and attL2 reacts only with attR2. Likewise, attB1 reacts only with attP1, and attB2 reacts only with attP2. Thus, the invention also relates to methods of controlled or directional cloning using the recombination sites of the invention (or portions thereof), including variants, fragments, mutants and derivatives thereof which may have altered or enhanced specificity. The invention also relates more generally to any number of recombination site partners or pairs (where each recombination site is specific for and interacts with its corresponding recombination site). Such recombination sites are preferably made by mutating or modifying the recombination site to provide any number of necessary specificities (e.g., attB1-10, attP1-10, attL1-10, attR1-10, etc.), non-limiting examples of which are described in detail in the Examples herein.

When an aliquot from the recombination reaction is transformed into host cells (e.g., *E. coli*) and spread on plates containing an appropriate selection agent, e.g., an antibiotic such as ampicillin with or without methicillin, cells that take up the desired clone form colonies. The unreacted Destination Vector does not give ampicillin-resistant colonies, even though it carries the ampicillin-resistance gene, because it contains a toxic gene, e.g., *ccdB*. Thus selection for ampicillin resistance selects for *E. coli* cells that carry the desired product, which usually comprise >90% of the colonies on the ampicillin plate.

To participate in the Recombinational (or “GATEWAY™”) Cloning Reaction, a nucleic acid molecule of interest first may be cloned into an Entry

Vector, creating an Entry Clone. Multiple options are available for creating Entry Clones, including: cloning of PCR sequences with terminal attB recombination sites into Entry Vectors; using the GATEWAY™ Cloning System recombination reaction; transfer of genes from libraries prepared in GATEWAY™ Cloning System vectors by recombination into Entry Vectors; and cloning of restriction enzyme-generated fragments and PCR fragments into Entry Vectors by standard recombinant DNA methods. These approaches are discussed in further detail herein.

A key advantage of the GATEWAY™ Cloning System is that a nucleic acid molecule of interest (or even a population of nucleic acid molecules of interest) present as an Entry Clone can be subcloned in parallel into one or more Destination Vectors in a simple reactions for anywhere from about 30 seconds to about 60 minutes (preferably about 1-60 minutes, about 1-45 minutes, about 1-30 minutes, about 2-60 minutes, about 2-45 minutes, about 2-30 minutes, about 1-2 minutes, about 30-60 minutes, about 45-60 minutes, or about 30-45 minutes). Longer reaction times (*e.g.*, 2-24 hours, or overnight) may increase recombination efficiency, particularly where larger nucleic acid molecules are used, as described in the Examples herein. Moreover, a high percentage of the colonies obtained carry the desired Expression Clone. This process is illustrated schematically in Figure 3, which shows an advantage of the invention in which the molecule of interest can be moved simultaneously or separately into multiple Destination Vectors. In the LR Reaction, one or both of the nucleic acid molecules to be recombined may have any topology (*e.g.*, linear, relaxed circular, nicked circular, supercoiled, etc.), although one or both are preferably linear.

The second major pathway of the GATEWAY™ Cloning System is the **BP Reaction** (Figure 4), which may also be referred to interchangeably herein as the **Entry Reaction** or the **Gateward Reaction**. The BP Reaction may recombine an Expression Clone with a Donor Plasmid (the counterpart of the byproduct in Figure 2). This reaction transfers the nucleic acid molecule of interest (which may have any of a variety of topologies, including linear, coiled, supercoiled, etc.) in the Expression Clone into an Entry Vector, to produce a new Entry Clone. Once this nucleic acid molecule of interest is cloned into an Entry

Vector, it can be transferred into new Expression Vectors, through the LR Reaction as described above. In the BP Reaction, one or both of the nucleic acid molecules to be recombined may have any topology (*e.g.*, linear, relaxed circular, nicked circular, supercoiled, etc.), although one or both are preferably linear.

5 A useful variation of the BP Reaction permits rapid cloning and expression of products of amplification (*e.g.*, PCR) or nucleic acid synthesis. Amplification (*e.g.*, PCR) products synthesized with primers containing terminal 25 bp attB sites serve as efficient substrates for the Gateway Cloning reaction. Such amplification products may be recombined with a Donor Vector to produce an Entry Clone (see
10 Figure 7). The result is an Entry Clone containing the amplification fragment. Such Entry Clones can then be recombined with Destination Vectors -- through the LR Reaction -- to yield Expression Clones of the PCR product.

 Additional details of the LR Reaction are shown in Figure 5A. The GATEWAY™ LR Clonase™ Enzyme Mix that mediates this reaction contains
15 lambda recombination proteins Int (Integrase), Xis (Excisionase), and IHF (Integration Host Factor). In contrast, the GATEWAY™ BP Clonase™ Enzyme Mix, which mediates the BP Reaction (Figure 5B), comprises Int and IHF alone.

 The recombination (att) sites of each vector comprise two distinct segments, donated by the parental vectors. The staggered lines dividing the two
20 portions of each att site, depicted in Figures 5A and 5B, represent the seven-base staggered cut produced by Int during the recombination reactions. This structure is seen in greater detail in Figure 6, which displays the attB recombination sequences of an Expression Clone, generated by recombination between the attL1 and attL2 sites of an Entry Clone and the attR1 and attR2 sites of a Destination
25 Vector.

 The nucleic acid molecule of interest in the Expression Clone is flanked by attB sites: attB1 to the left (amino terminus) and attB2 to the right (carboxy terminus). The bases in attB1 to the left of the seven-base staggered cut produced by Int are derived from the Destination vector, and the bases to the right of the
30 staggered cut are derived from the Entry Vector (see Figure 6). Note that the sequence is displayed in triplets corresponding to an open reading frame. If the reading frame of the nucleic acid molecule of interest cloned in the Entry Vector

is in phase with the reading frame shown for attB1, amino-terminal protein fusions can be made between the nucleic acid molecule of interest and any GATEWAY™ Cloning System Destination Vector encoding an amino-terminal fusion domain. Entry Vectors and Destination Vectors that enable cloning in all three reading frames are described in more detail herein, particularly in the Examples.

The LR Reaction allows the transfer of a desired nucleic acid molecule of interest into new Expression Vectors by recombining a Entry Clone with various Destination Vectors. To participate in the LR or Destination Reaction, however, a nucleic acid molecule of interest preferably is first converted to a Entry Clone. Entry Clones can be made in a number of ways, as shown in Figure 7.

One approach is to clone the nucleic acid molecule of interest into one or more of the Entry Vectors, using standard recombinant DNA methods, with restriction enzymes and ligase. The starting DNA fragment can be generated by restriction enzyme digestion or as a PCR product. The fragment is cloned between the attL1 and attL2 recombination sites in the Entry Vector. Note that a toxic or “death” gene (*e.g.*, *ccdB*), provided to minimize background colonies from incompletely digested Entry Vector, must be excised and replaced by the nucleic acid molecule of interest.

A second approach to making an Entry Clone (Figure 7) is to make a library (genomic or cDNA) in an Entry Vector, as described in detail herein. Such libraries may then be transferred into Destination Vectors for expression screening, for example in appropriate host cells such as yeast cells or mammalian cells.

A third approach to making Entry Clones (Figure 7) is to use Expression Clones obtained from cDNA molecules or libraries prepared in Expression Vectors. Such cDNAs or libraries, flanked by attB sites, can be introduced into a Entry Vector by recombination with a Donor Vector via the BP Reaction. If desired, an entire Expression Clone library can be transferred into the Entry Vector through the BP Reaction. Expression Clone cDNA libraries may also be constructed in a variety of prokaryotic and eukaryotic GATEWAY™-modified vectors (*e.g.*, the pEXP501 Expression Vector (see Figure 48), and 2-hybrid and

attB library vectors), as described in detail herein, particularly in the Examples below.

A fourth, and potentially most versatile, approach to making an Entry Clone (Figure 7) is to introduce a sequence for a nucleic acid molecule of interest into an Entry Vector by amplification (*e.g.*, PCR) fragment cloning. This method is diagramed in Figure 8. The DNA sequence first is amplified (for example, with PCR) as outlined in detail below and in the Examples herein, using primers containing one or more bp, two or more bp, three or more bp, four or more bp, five or more bp, preferably six or more bp, more preferably 6-25 bp (particularly 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25) bp of the attB nucleotide sequences (such as, but not limited to, those depicted in Figure 9), and optionally one or more, two or more, three or more, four or more, and most preferably four or five or more additional terminal nucleotide bases which preferably are guanines. The PCR product then may be converted to a Entry Clone by performing a BP Reaction, in which the attB-PCR product recombines with a Donor Vector containing one or more attP sites. Details of this approach and protocols for PCR fragment subcloning are provided in Examples 8 and 21-25.

A variety of Entry Clones may be produced by these methods, providing a wide array of cloning options; a number of specific Entry Vectors are also available commercially from Life Technologies, Inc. (Rockville, MD). The Examples herein provide a more in-depth description of selected Entry Vectors and details of their cloning sites. Choosing the optimal Entry Vector for a particular application is discussed in Example 4.

Entry Vectors and Destination Vectors should be constructed so that the amino-terminal region of a nucleic acid molecule of interest (*e.g.*, a gene, cDNA library or insert, or fragment thereof) will be positioned next to the attL1 site. Entry Vectors preferably contain the *rrnB* transcriptional terminator upstream of the attL1 site. This sequence ensures that expression of cloned nucleic acid molecules of interest is reliably "off" in *E. coli*, so that even toxic genes can be successfully cloned. Thus, Entry Clones may be designed to be transcriptionally silent. Note also that Entry Vectors, and hence Entry Clones, may contain the kanamycin antibiotic resistance (*kan*^r) gene to facilitate selection of host cells

containing Entry Clones after transformation. In certain applications, however, Entry Clones may contain other selection markers, including but not limited to a gentamycin resistance (*gen^r*) or tetracycline resistance (*tet^r*) gene, to facilitate selection of host cells containing Entry Clones after transformation.

5 Once a nucleic acid molecule of interest has been cloned into an Entry Vector, it may be moved into a Destination Vector. The upper right portion of Figure 5A shows a schematic of a Destination Vector. The thick arrow represents some function (often transcription or translation) that will act on the nucleic acid molecule of interest in the clone. During the recombination reaction, the region
10 between the *attR1* and *attR2* sites, including a toxic or "death" gene (*e.g.*, *ccdB*), is replaced by the DNA segment from the Entry Clone. Selection for recombinants that have acquired the ampicillin resistance (*amp^r*) gene (carried on the Destination Vector) and that have also lost the death gene ensures that a high percentage (usually >90%) of the resulting colonies will contain the correct insert.

15 To move a nucleic acid molecule of interest into a Destination Vector, the Destination Vector is mixed with the Entry Clone comprising the desired nucleic acid molecule of interest, a cocktail of recombination proteins (*e.g.*, GATEWAY™ LR Clonase™ Enzyme Mix) is added, the mixture is incubated (preferably at about 25°C for about 60 minutes, or longer under certain
20 circumstances, *e.g.* for transfer of large nucleic acid molecules, as described below) and any standard host cell (including bacterial cells such as *E. coli*; animal cells such as insect cells, mammalian cells, nematode cells and the like; plant cells; and yeast cells) strain is transformed with the reaction mixture. The host cell used will be determined by the desired selection (*e.g.*, *E. coli* DB3.1, available
25 commercially from Life Technologies, Inc., allows survival of clones containing the *ccdB* death gene, and thus can be used to select for cointegrate molecules -- *i.e.*, molecules that are hybrids between the Entry Clone and Destination Vector). The Examples below provide further details and protocols for use of Entry and Destination Vectors in transferring nucleic acid molecules of interest and
30 expressing RNAs or polypeptides encoded by these nucleic acid molecules in a variety of host cells.

The cloning system of the invention therefore offers multiple advantages:

- Once a nucleic acid molecule of interest is cloned into the GATEWAY™ Cloning System, it can be moved into and out of other vectors with complete fidelity of reading frame and orientation. That is, since the reactions proceed whereby attL1 on the Entry Clone recombines with attR1 on the Destination Vector, the directionality of the nucleic acid molecule of interest is maintained or may be controlled upon transfer from the Entry Clone into the Destination Vector. Hence, the GATEWAY™ Cloning System provides a powerful and easy method of directional cloning of nucleic acid molecule of interest.
- One-step cloning or subcloning: Mix the Entry Clone and the Destination Vector with Clonase, incubate, and transform.
- Clone PCR products readily by *in vitro* recombination, by adding attB sites to PCR primers. Then directly transfer these Entry Clones into Destination Vectors. This process may also be carried out in one step (see Examples below).
- Powerful selections give high reliability: >90% (and often >99%) of the colonies contain the desired DNA in its new vector.
- One-step conversion of existing standard vectors into GATEWAY™ Cloning System vectors.
- Ideal for large vectors or those with few cloning sites.
- Recombination sites are short (25 bp), and may be engineered to contain no stop codons or secondary structures.
- Reactions may be automated, for high-throughput applications (*e.g.*, for diagnostic purposes or for therapeutic candidate screening).
- The reactions are economical: 0.3 µg of each DNA; no restriction enzymes, phosphatase, ligase, or gel purification. Reactions work well with miniprep DNA.
- Transfer multiple clones, and even libraries, into one or more Destination Vectors, in a single experiment.
- A variety of Destination Vectors may be produced, for applications including, but not limited to:

- Protein expression in *E. coli*: native proteins; fusion proteins with GST, His6, thioredoxin, etc., for purification, or one or more epitope tags; any promoter useful in expressing proteins in *E. coli* may be used, such as ptrc, λP_L , and T7 promoters.
- Protein expression in eukaryotic cells: CMV promoter, baculovirus (with or without His6 tag), Semliki Forest virus, Tet regulation.
- DNA sequencing (all *lac* primers), RNA probes, phagemids (both strands)
- A variety of Entry Vectors (for recombinational cloning entry by standard recombinant DNA methods) may be produced:
 - Strong transcription stop just upstream, for genes toxic to *E. coli*.
 - Three reading frames.
 - With or without TEV protease cleavage site.
 - Motifs for prokaryotic and / or eukaryotic translation.
 - Compatible with commercial cDNA libraries.
- Expression Clone cDNA (*attB*) libraries, for expression screening, including 2-hybrid libraries and phage display libraries, may also be constructed.

Recombination Site Sequences

In one aspect, the invention relates to nucleic acid molecules, which may or may not be isolated nucleic acid molecules, comprising one or more nucleotide sequences encoding one or more recombination sites or portions thereof. In particular, this aspect of the invention relates to such nucleic acid molecules comprising one or more nucleotide sequences encoding *attB*, *attP*, *attL*, or *attR*, or portions of these recombination site sequences. The invention also relates to mutants, derivatives, and fragments of such nucleic acid molecules. Unless otherwise indicated, all nucleotide sequences that may have been determined by sequencing a DNA molecule herein were determined using manual or automated DNA sequencing, such as dideoxy sequencing, according to methods that are routine to one of ordinary skill in the art (Sanger, F., and Coulson, A.R., *J. Mol. Biol.* 94:444-448 (1975); Sanger, F., *et al.*, *Proc. Natl. Acad. Sci. USA* 74:5463-5467 (1977)). All amino acid sequences of polypeptides encoded by DNA

molecules determined herein were predicted by conceptual translation of a DNA sequence determined as above. Therefore, as is known in the art for any DNA sequence determined by these approaches, any nucleotide sequence determined herein may contain some errors. Nucleotide sequences determined by such methods are typically at least about 90% identical, more typically at least about 95% to at least about 99.9% identical to the actual nucleotide sequence of the sequenced DNA molecule. As is also known in the art, a single insertion or deletion in a determined nucleotide sequence compared to the actual sequence will cause a frame shift in translation of the nucleotide sequence such that the predicted amino acid sequence encoded by a determined nucleotide sequence will be completely different from the amino acid sequence actually encoded by the sequenced DNA molecule, beginning at the point of such an insertion or deletion.

Unless otherwise indicated, each "nucleotide sequence" set forth herein is presented as a sequence of deoxyribonucleotides (abbreviated A, G, C and T). However, by "nucleotide sequence" of a nucleic acid molecule or polynucleotide is intended, for a DNA molecule or polynucleotide, a sequence of deoxyribonucleotides, and for an RNA molecule or polynucleotide, the corresponding sequence of ribonucleotides (A, G, C and U), where each thymidine deoxyribonucleotide (T) in the specified deoxyribonucleotide sequence is replaced by the ribonucleotide uridine (U). Thus, the invention relates to sequences of the invention in the form of DNA or RNA molecules, or hybrid DNA/RNA molecules, and their corresponding complementary DNA, RNA, or DNA/RNA strands.

In a first such aspect, the invention provides nucleic acid molecules comprising one or more nucleotide sequences encoding *attB1*, or mutants, fragments, variants or derivatives thereof. Such nucleic acid molecules may comprise an *attB1* nucleotide sequence having the sequence set forth in Figure 9, such as: ACAAGTTTGTACAAAAAAGCAGGCT, or a nucleotide sequence complementary to the nucleotide sequence set forth in Figure 9 for *attB1*, or mutants, fragments, variants or derivatives thereof. As one of ordinary skill will appreciate, however, certain mutations, insertions, or deletions of one or more bases in the *attB1* sequence contained in the nucleic acid molecules of the invention may be made without compromising the structural and functional

integrity of these molecules; hence, nucleic acid molecules comprising such mutations, insertions, or deletions in the *attB1* sequence are encompassed within the scope of the invention.

In a related aspect, the invention provides nucleic acid molecules comprising one or more nucleotide sequences encoding *attB2*, or mutants, fragments, variants or derivatives thereof. Such nucleic acid molecules may comprise an *attB2* nucleotide sequence having the sequence set forth in Figure 9, such as: ACCCAGCTTTCTTGTACAAAGTGGT, or a nucleotide sequence complementary to the nucleotide sequence set forth in Figure 9 for *attB2*, or mutants, fragments, variants or derivatives thereof. As noted above for *attB1*, certain mutations, insertions, or deletions of one or more bases in the *attB2* sequence contained in the nucleic acid molecules of the invention may be made without compromising the structural and functional integrity of these molecules; hence, nucleic acid molecules comprising such mutations, insertions, or deletions in the *attB2* sequence are encompassed within the scope of the invention.

A recombinant host cell comprising a nucleic acid molecule containing *attB1* and *attB2* sites (the vector pEXP501, also known as pCMVSPORT6; see Figure 48), *E. coli* DB3.1(pCMVSPORT6), was deposited on February 27, 1999, with the Collection, Agricultural Research Culture Collection (NRRL), 1815 North University Street, Peoria, Illinois 61604 USA, as Deposit No. NRRL B-30108. The *attB1* and *attB2* sites within the deposited nucleic acid molecule are contained in nucleic acid cassettes in association with one or more additional functional sequences as described in more detail below.

In another related aspect, the invention provides nucleic acid molecules comprising one or more nucleotide sequences encoding *attP1*, or mutants, fragments, variants or derivatives thereof. Such nucleic acid molecules may comprise an *attP1* nucleotide sequence having the sequence set forth in Figure 9, such as: TACAGGTCACCTAATACCATCTAAGTAGTTGATTCATAGTGA-CTGGATATGTTGTGTTTTACAGTATTATGTAGTCTGTTTTTTAT-GCAAAATCTAATTTAATATATTGATATTTATATCATTTTACGTT-TCTCGTTCAGCTTTTTTGTACAAAGTTGGCATTATAAAAAAGCATTG-CTCATCAATTTGTTGCAACGAACAGGTCCTATCAGTCAAAATAA-

AATCATTATTTG, or a nucleotide sequence complementary to the nucleotide sequence set forth in Figure 9 for *attP1*, or mutants, fragments, variants or derivatives thereof. As noted above for *attB1*, certain mutations, insertions, or deletions of one or more bases in the *attP1* sequence contained in the nucleic acid molecules of the invention may be made without compromising the structural and functional integrity of these molecules; hence, nucleic acid molecules comprising such mutations, insertions, or deletions in the *attP1* sequence are encompassed within the scope of the invention.

In another related aspect, the invention provides nucleic acid molecules comprising one or more nucleotide sequences encoding *attP2*, or mutants, fragments, variants or derivatives thereof. Such nucleic acid molecules may comprise an *attP2* nucleotide sequence having the sequence set forth in Figure 9, such as: CAAATAATGATTTTATTTTGACTGATAGTGACCTGTTCGTTG-CAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTT-GTACAAGAAAGCTGAACGAGAAACGTAAAATGATA-TAAATATCAATATATTAAATTAGATTTTGCATAAAAAACAG-ACTACATAATACTGTAAAACACAACATATCCAGTCACTATGAATCAACTACTTAGATGGTATTAGTGACCTGTA, or a nucleotide sequence complementary to the nucleotide sequence set forth in Figure 9 for *attP2*, or mutants, fragments, variants or derivatives thereof. As noted above for *attB1*, certain mutations, insertions, or deletions of one or more bases in the *attP2* sequence contained in the nucleic acid molecules of the invention may be made without compromising the structural and functional integrity of these molecules; hence, nucleic acid molecules comprising such mutations, insertions, or deletions in the *attP2* sequence are encompassed within the scope of the invention.

A recombinant host cell comprising a nucleic acid molecule (the attP vector pDONR201, also known as pENTR21-attPkan or pAttPkan; see Figure 49) containing attP1 and attP2 sites, *E. coli* DB3.1(pAttPkan) (also called *E. coli* DB3.1(pAHKAn)), was deposited on February 27, 1999, with the Collection, Agricultural Research Culture Collection (NRRL), 1815 North University Street, Peoria, Illinois 61604 USA, as Deposit No. NRRL B-30099. The attP1 and attP2 sites within the deposited nucleic acid molecule are contained in nucleic acid

cassettes in association with one or more additional functional sequences as described in more detail below.

In another related aspect, the invention provides nucleic acid molecules comprising one or more nucleotide sequences encoding *attR1*, or mutants, fragments, variants or derivatives thereof. Such nucleic acid molecules may comprise an *attR1* nucleotide sequence having the sequence set forth in Figure 9, such as: A C A A G T T T G T A C A A A A A G C T G A A C G A G -
AAACGTAAAATGATATAAATATCAATATATTAAATTAGATTTTGCAT-
AAAAAACAGACTACATAATACTGTAAAACACAACATATCCAGTCA-
CTATG, or a nucleotide sequence complementary to the nucleotide sequence set forth in Figure 9 for *attR1*, or mutants, fragments, variants or derivatives thereof. As noted above for *attB1*, certain mutations, insertions, or deletions of one or more bases in the *attR1* sequence contained in the nucleic acid molecules of the invention may be made without compromising the structural and functional integrity of these molecules; hence, nucleic acid molecules comprising such mutations, insertions, or deletions in the *attR1* sequence are encompassed within the scope of the invention.

In another related aspect, the invention provides nucleic acid molecules comprising one or more nucleotide sequences encoding *attR2*, or mutants, fragments, variants or derivatives thereof. Such nucleic acid molecules may comprise an *attR2* nucleotide sequence having the sequence set forth in Figure 9, such as: G C A G G T C G A C C A T A G T G A C T G G A T A T -
GTTGTGTTTTACAGTATTATGTAGTCTGTTTTTTATGCAAAATCTA-
ATTTAATATATTGATATTTATATCATTTTACGTTTCTCGTTTCAGCTT-
TCTTGTACAAAGTGGT, or a nucleotide sequence complementary to the nucleotide sequence set forth in Figure 9 for *attR2*, or mutants, fragments, variants or derivatives thereof. As noted above for *attB1*, certain mutations, insertions, or deletions of one or more bases in the *attR2* sequence contained in the nucleic acid molecules of the invention may be made without compromising the structural and functional integrity of these molecules; hence, nucleic acid molecules comprising such mutations, insertions, or deletions in the *attR2* sequence are encompassed within the scope of the invention.

Recombinant host cell strains containing attR1 sites apposed to cloning sites in reading frame A, reading frame B, and reading frame C, *E. coli* DB3.1(pEZC15101) (reading frame A; see Figure 64A), *E. coli* DB3.1(pEZC15102) (reading frame B; see Figure 64B), and *E. coli* DB3.1(pEZC15103) (reading frame C; see Figure 64C), and containing corresponding attR2 sites, were deposited on February 27, 1999, with the Collection, Agricultural Research Culture Collection (NRRL), 1815 North University Street, Peoria, Illinois 61604 USA, as Deposit Nos. NRRL B-30103, NRRL B-30104, and NRRL B-30105, respectively. The attR1 and attR2 sites within the deposited nucleic acid molecules are contained in nucleic acid cassettes in association with one or more additional functional sequences as described in more detail below.

In another related aspect, the invention provides nucleic acid molecules comprising one or more nucleotide sequences encoding attL1, or mutants, fragments, variants and derivatives thereof. Such nucleic acid molecules may comprise an attL1 nucleotide sequence having the sequence set forth in Figure 9, such as: CAA ATA ATG ATT TTA TTT TGA CTG ATA GTG ACC TGT TCG TTG CAA CAA ATT GAT AAG CAA TGC TTT TTT ATA ATG CCA ACT TTG TAC AAA AAA GCA GGC T, or a nucleotide sequence complementary to the nucleotide sequence set forth in Figure 9 for attL1, or mutants, fragments, variants or derivatives thereof. As noted above for attB1, certain mutations, insertions, or deletions of one or more bases in the attL1 sequence contained in the nucleic acid molecules of the invention may be made without compromising the structural and functional integrity of these molecules; hence, nucleic acid molecules comprising such mutations, insertions, or deletions in the attL1 sequence are encompassed within the scope of the invention.

In another related aspect, the invention provides nucleic acid molecules comprising one or more nucleotide sequences encoding attL2, or mutants, fragments, variants and derivatives thereof. Such nucleic acid molecules may comprise an attL2 nucleotide sequence having the sequence set forth in Figure 9, such as: C AAA TAA TGA TTT TAT TTT GAC TGA TAG TGA CCT GTT CGT TGC AAC AAA TTG ATA AGC AAT GCT TTC TTA TAA TGC CAA

CTT TGT ACA AGA AAG CTG GGT, or a nucleotide sequence complementary to the nucleotide sequence set forth in Figure 9 for *attL2*, or mutants, fragments, variants or derivatives thereof. As noted above for *attB1*, certain mutations, insertions, or deletions of one or more bases in the *attL2* sequence contained in the nucleic acid molecules of the invention may be made without compromising the structural and functional integrity of these molecules; hence, nucleic acid molecules comprising such mutations, insertions, or deletions in the *attL2* sequence are encompassed within the scope of the invention.

Recombinant host cell strains containing *attL1* sites apposed to cloning sites in reading frame A, reading frame B, and reading frame C, *E. coli* DB3.1(pENTR1A) (reading frame A; see Figure 10), *E. coli* DB3.1(pENTR2B) (reading frame B; see Figure 11), and *E. coli* DB3.1(pENTR3C) (reading frame C; see Figure 12), and containing corresponding *attL2* sites, were deposited on February 27, 1999, with the Collection, Agricultural Research Culture Collection (NRRL), 1815 North University Street, Peoria, Illinois 61604 USA, as Deposit Nos. NRRL B-30100, NRRL B-30101, and NRRL B-30102, respectively. The *attL1* and *attL2* sites within the deposited nucleic acid molecules are contained in nucleic acid cassettes in association with one or more additional functional sequences as described in more detail below.

Each of the recombination site sequences described herein or portions thereof, or the nucleotide sequence cassettes contained in the deposited clones, may be cloned or inserted into a vector of interest (for example, using the recombinational cloning methods described herein and/or standard restriction cloning techniques that are routine in the art) to generate, for example, Entry Vectors or Destination Vectors which may be used to transfer a desired segment of a nucleic acid molecule of interest (*e.g.*, a gene, cDNA molecule, or cDNA library) into a desired vector or into a host cell.

Using the information provided herein, such as the nucleotide sequences for the recombination site sequences described herein, an isolated nucleic acid molecule of the present invention encoding one or more recombination sites or portions thereof may be obtained using standard cloning and screening procedures, such as those for cloning cDNAs using mRNA as starting material. Preferred such

methods include PCR-based cloning methods, such as reverse transcriptase-PCR (RT-PCR) using primers such as those described herein and in the Examples below. Alternatively, vectors comprising the cassettes containing the recombination site sequences described herein are available commercially from Life Technologies, Inc. (Rockville, MD).

The invention is also directed to nucleic acid molecules comprising one or more of the recombination site sequences or portions thereof and one or more additional nucleotide sequences, which may encode functional or structural sites such as one or more multiple cloning sites, one or more transcription termination sites, one or more transcriptional regulatory sequences (which may be promoters, enhancers, repressors, and the like), one or more translational signals (*e.g.*, secretion signal sequences), one or more origins of replication, one or more fusion partner peptides (particularly glutathione S-transferase (GST), hexahistidine (His₆), and thioredoxin (Trx)), one or more selection markers or modules, one or more nucleotide sequences encoding localization signals such as nuclear localization signals or secretion signals, one or more origins of replication, one or more protease cleavage sites, one or more genes or portions of genes encoding a protein or polypeptide of interest, and one or more 5' polynucleotide extensions (particularly an extension of guanine residues ranging in length from about 1 to about 20, from about 2 to about 15, from about 3 to about 10, from about 4 to about 10, and most preferably an extension of 4 or 5 guanine residues at the 5' end of the recombination site nucleotide sequence. The one or more additional functional or structural sequences may or may not flank one or more of the recombination site sequences contained on the nucleic acid molecules of the invention.

In some nucleic acid molecules of the invention, the one or more nucleotide sequences encoding one or more additional functional or structural sites may be operably linked to the nucleotide sequence encoding the recombination site. For example, certain nucleic acid molecules of the invention may have a promoter sequence operably linked to a nucleotide sequence encoding a recombination site or portion thereof of the invention, such as a T7 promoter, a phage lambda PL

promoter, an *E. coli lac*, *trp* or *tac* promoter, and other suitable promoters which will be familiar to the skilled artisan.

Nucleic acid molecules of the present invention, which may be isolated nucleic acid molecules, may be in the form of RNA, such as mRNA, or in the form of DNA, including, for instance, cDNA and genomic DNA obtained by cloning or produced synthetically, or in the form of DNA-RNA hybrids. The nucleic acid molecules of the invention may be double-stranded or single-stranded. Single-stranded DNA or RNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand. The nucleic acid molecules of the invention may also have a number of topologies, including linear, circular, coiled, or supercoiled.

By "isolated" nucleic acid molecule(s) is intended a nucleic acid molecule, DNA or RNA, which has been removed from its native environment. For example, recombinant DNA molecules contained in a vector are considered isolated for the purposes of the present invention. Further examples of isolated DNA molecules include recombinant DNA molecules maintained in heterologous host cells, and those DNA molecules purified (partially or substantially) from a solution whether produced by recombinant DNA or synthetic chemistry techniques. Isolated RNA molecules include *in vivo* or *in vitro* RNA transcripts of the DNA molecules of the present invention.

The present invention further relates to mutants, fragments, variants and derivatives of the nucleic acid molecules of the present invention, which encode portions, analogs or derivatives of one or more recombination sites. Variants may occur naturally, such as a natural allelic variant. By an "allelic variant" is intended one of several alternate forms of a gene occupying a given locus on a chromosome of an organism (*see* Lewin, B., ed., *Genes II*, , John Wiley & Sons, New York (1985)). Non-naturally occurring variants may be produced using art-known mutagenesis techniques, such as those described hereinbelow.

Such variants include those produced by nucleotide substitutions, deletions or additions or portions thereof, or combinations thereof. The substitutions, deletions or additions may involve one or more nucleotides. The variants may be altered in coding regions, non-coding regions, or both. Alterations in the coding

regions may produce conservative or non-conservative amino acid substitutions, deletions or additions. Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of the encoded polypeptide(s) or portions thereof, and which also do not substantially alter the reactivities of the recombination site nucleic acid sequences in recombination reactions. Also especially preferred in this regard are conservative substitutions.

Particularly preferred mutants, fragments, variants, and derivatives of the nucleic acid molecules of the invention include, but are not limited to, insertions, deletions or substitutions of one or more nucleotide bases within the 15 bp core region (GCTTTTTTATACTAA) which is identical in all four wildtype lambda *att* sites, *attB*, *attP*, *attL* and *attR* (see U.S. Application Nos. 08/663,002, filed June 7, 1996 (now U.S. Patent No. 5,888,732), 09/005,476, filed January 12, 1998, and 09/177,387, filed October 23, 1998, which describes the core region in further detail, and the disclosures of which are incorporated herein by reference in their entireties). Analogously, the core regions in *attB*1, *attP*1, *attL*1 and *attR*1 are identical to one another, as are the core regions in *attB*2, *attP*2, *attL*2 and *attR*2. Particularly preferred in this regard are nucleic acid molecules comprising insertions, deletions or substitutions of one or more nucleotides within the seven bp overlap region (TTTATAC, which is defined by the cut sites for the integrase protein and is the region where strand exchange takes place) that occurs within this 15 bp core region (GCTTTTTTTTTATACTAA). Examples of such preferred mutants, fragments, variants and derivatives according to this aspect of the invention include, but are not limited to, nucleic acid molecules in which the thymine at position 1 of the seven bp overlap region has been deleted or substituted with a guanine, cytosine, or adenine; in which the thymine at position 2 of the seven bp overlap region has been deleted or substituted with a guanine, cytosine, or adenine; in which the thymine at position 3 of the seven bp overlap region has been deleted or substituted with a guanine, cytosine, or adenine; in which the adenine at position 4 of the seven bp overlap region has been deleted or substituted with a guanine, cytosine, or thymine; in which the thymine at position 5 of the seven bp overlap region has been deleted or substituted with a

guanine, cytosine, or adenine; in which the adenine at position 6 of the seven bp overlap region has been deleted or substituted with a guanine, cytosine, or thymine; and in which the cytosine at position 7 of the seven bp overlap region has been deleted or substituted with a guanine, thymine, or adenine; or any combination of one or more such deletions and/or substitutions within this seven bp overlap region. As described in detail in Example 21 herein, mutants of the nucleic acid molecules of the invention in which substitutions have been made within the first three positions of the seven bp overlap (TTTTATAC) have been found in the present invention to strongly affect the specificity of recombination, mutant nucleic acid molecules in which substitutions have been made in the last four positions (TTTTATAC) only partially alter recombination specificity, and mutant nucleic acid molecules comprising nucleotide substitutions outside of the seven bp overlap, but elsewhere within the 15 bp core region, do not affect specificity of recombination but do influence the efficiency of recombination.

Hence, in an additional aspect, the present invention is also directed to nucleic acid molecules comprising one or more recombination site nucleotide sequences that affect recombination specificity, particularly one or more nucleotide sequences that may correspond substantially to the seven base pair overlap within the 15 bp core region, having one or more mutations that affect recombination specificity. Particularly preferred such molecules may comprise a consensus sequence (described in detail in Example 21 herein) such as NNNATAC, wherein "N" refers to any nucleotide (*i.e.*, may be A, G, T/U or C), with the proviso that if one of the first three nucleotides in the consensus sequence is a T/U, then at least one of the other two of the first three nucleotides is not a T/U.

In a related aspect, the present invention is also directed to nucleic acid molecules comprising one or more recombination site nucleotide sequences that enhance recombination efficiency, particularly one or more nucleotide sequences that may correspond substantially to the core region and having one or more mutations that enhance recombination efficiency. By sequences or mutations that "enhance recombination efficiency" is meant a sequence or mutation in a recombination site, preferably in the core region (*e.g.*, the 15 bp core region of *att* recombination sites), that results in an increase in cloning efficiency (typically

measured by determining successful cloning of a test sequence, *e.g.*, by determining CFU/ml for a given cloning mixture) when recombining molecules comprising the mutated sequence or core region as compared to molecules that do not comprise the mutated sequence or core region (*e.g.*, those comprising a wildtype recombination site core region sequence). More specifically, whether or not a given sequence or mutation enhances recombination efficiency may be determined using the sequence or mutation in recombinational cloning as described herein, and determining whether the sequence or mutation provides enhanced recombinational cloning efficiency when compared to a non-mutated (*e.g.*, wildtype) sequence. Methods of determining preferred cloning efficiency-enhancing mutations for a number of recombination sites, particularly for *att* recombination sites, are described herein, for example in Examples 22-25. Examples of preferred such mutant recombination sites include but are not limited to the *attL* consensus core sequence of caactnntnnnannaagttg (wherein "n" represents any nucleotide), for example the *attL5* sequence agcctgctttattataactaagttggcatta and the *attL6* sequence agcctgcttttttatattaagttggcatta; the *attB1.6* sequence ggggacaactttgtacaaaaaagttggct; the *attB2.2* sequence ggggacaactttgtacaagaaagctgggt; and the *attB2.10* sequence ggggacaactttgtacaagaaagttgggt. Those of skill in the art will appreciate that, in addition to the core region, other portions of the *att* site may affect the efficiency of recombination. There are five so-called arm binding sites for the integrase protein in the bacteriophage lambda *attP* site, two in *attR* (P1 and P2), and three in *attL* (P'1, P'2 and P'3). Compared to the core binding sites, the integrase protein binds to arm sites with high affinity and interacts with core and arm sites through two different domains of the protein. As with the core binding site a consensus sequence for the arm binding site consisting of C/AAGTCACTAT has been inferred from sequence comparison of the five arm binding sites and seven non-*att* sites (Ross and Landy, *Proc. Natl. Acad. Sci. USA* 79:7724-7728 (1982)). Each arm site has been mutated and tested for its effect in the excision and integration reactions (Numrych *et al.*, *Nucl. Acids Res.* 18:3953 (1990)). Hence, specific sites are utilized in each reaction in different ways, namely, the P1 and P'3

sites are essential for the integration reaction whereas the other three sites are dispensable to the integration reaction to varying degrees. Similarly, the P2, P'1 and P'2 sites are most important for the excision reaction, whereas P1 and P'3 are completely dispensable. Interestingly, when P2 is mutated the integration reaction occurs more efficiently than with the wild type attP site. Similarly, when P1 and P'3 are mutated the excision reaction occurs more efficiently. The stimulatory effect of mutating integrase arm binding sites can be explained by removing sites that compete or inhibit a specific recombination pathway or that function in a reaction that converts products back to starting substrates. In fact there is evidence for an XIS-independent LR reaction (Abremski and Gottesman, *J. Mol. Biol.* 153:67-78 (1981)). Thus, in addition to modifications in the core region of the att site, the present invention contemplates the use of att sites containing one or more modifications in the integrase arm-type binding sites. In some preferred embodiments, one or more mutations may be introduced into one or more of the P1, P'1, P2, P'2 and P'3 sites. In some preferred embodiments, multiple mutations may be introduced into one or more of these sites. Preferred such mutations include those which increase the recombination *in vitro*. For example, in some embodiments mutations may be introduced into the arm-type binding sites such that integrative recombination, corresponding to the BP reaction, is enhanced. In other embodiments, mutations may be introduced into the arm-type binding sites such that excisive recombination, corresponding to the LR reaction, is enhanced. Of course, based on the guidance contained herein, particularly in the construction and evaluation of effects of mutated recombination sites upon recombinational specificity and efficiency, analogous mutated or engineered sequences may be produced for other recombination sites described herein (including but not limited to *lox*, FRT, and the like) and used in accordance with the invention. For example, much like the mutagenesis strategy used to select core binding sites that enhance recombination efficiency, similar strategies can be employed to select changes in the arms of attP, attL and attR, and in analogous sequences in other recombination sites such as *lox*, FRT and the like, that enhance recombination efficiency. Hence, the construction and evaluation of such mutants is well within the abilities of those of ordinary skill in the art without undue experimentation.

One suitable methodology for preparing and evaluating such mutations is found in Numrych, *et al.*, (1990) *Nucleic Acids Research* 18(13): 3953-3959.

Other mutant sequences and nucleic acid molecules that may be suitable to enhance recombination efficiency will be apparent from the description herein, or may be easily determined by one of ordinary skill using only routine experimentation in molecular biology in view of the description herein and information that is readily available in the art

Since the genetic code is well known in the art, it is also routine for one of ordinary skill in the art to produce degenerate variants of the nucleic acid molecules described herein without undue experimentation. Hence, nucleic acid molecules comprising degenerate variants of nucleic acid sequences encoding the recombination sites described herein are also encompassed within the scope of the invention.

Further embodiments of the invention include isolated nucleic acid molecules comprising a polynucleotide having a nucleotide sequence at least 50% identical, at least 60% identical, at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, and more preferably at least 95%, 96%, 97%, 98% or 99% identical to the nucleotide sequences of the seven bp overlap region within the 15 bp core region of the recombination sites described herein, or the nucleotide sequences of *attB1*, *attB2*, *attP1*, *attP2*, *attL1*, *attL2*, *attR1* or *attR2* as set forth in Figure 9 (or portions thereof), or a nucleotide sequence complementary to any of these nucleotide sequences, or fragments, variants, mutants, and derivatives thereof.

By a polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence encoding a particular recombination site or portion thereof is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations (*e.g.*, insertions, substitutions, or deletions) per each 100 nucleotides of the reference nucleotide sequence encoding the recombination site. For example, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference *attB1* nucleotide sequence, up to 5% of the nucleotides in the *attB1* reference sequence may be

deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the *attB1* reference sequence may be inserted into the *attB1* reference sequence. These mutations of the reference sequence may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or
5 anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular nucleic acid molecule is at least 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical
10 to, for instance, a given recombination site nucleotide sequence or portion thereof can be determined conventionally using known computer programs such as DNAsis software (Hitachi Software, San Bruno, California) for initial sequence alignment followed by ESEE version 3.0 DNA/protein sequence software (cabot@trog.mbb.sfu.ca) for multiple sequence alignments. Alternatively, such
15 determinations may be accomplished using the BESTFIT program (Wisconsin Sequence Analysis Package, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711), which employs a local homology algorithm (Smith and Waterman, *Advances in Applied Mathematics* 2: 482-489 (1981)) to find the best segment of homology between two sequences. When
20 using DNAsis, ESEE, BESTFIT or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set such that the percentage of identity is calculated over the full length of the reference nucleotide sequence and that gaps in homology of up to 5% of the total number
25 of nucleotides in the reference sequence are allowed.

The present invention is directed to nucleic acid molecules at least 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the *attB1*, *attB2*, *attP1*, *attP2*, *attL1*, *attL2*, *attR1* or *attR2* nucleotide sequences as set forth in Figure 9, or to the nucleotide sequence of the deposited clones, irrespective of
30 whether they encode particular functional polypeptides. This is because even where a particular nucleic acid molecule does not encode a particular functional polypeptide, one of skill in the art would still know how to use the nucleic acid

molecule, for instance, as a hybridization probe or a polymerase chain reaction (PCR) primer.

5 Mutations can also be introduced into the recombination site nucleotide sequences for enhancing site specific recombination or altering the specificities of the reactants, etc. Such mutations include, but are not limited to: recombination sites without translation stop codons that allow fusion proteins to be encoded; recombination sites recognized by the same proteins but differing in base sequence such that they react largely or exclusively with their homologous partners allowing multiple reactions to be contemplated; and mutations that prevent hairpin
10 formation of recombination sites. Which particular reactions take place can be specified by which particular partners are present in the reaction mixture.

There are well known procedures for introducing specific mutations into nucleic acid sequences. A number of these are described in Ausubel, F.M. *et al.*, *Current Protocols in Molecular Biology*, Wiley Interscience, New York (1989-
15 1996). Mutations can be designed into oligonucleotides, which can be used to modify existing cloned sequences, or in amplification reactions. Random mutagenesis can also be employed if appropriate selection methods are available to isolate the desired mutant DNA or RNA. The presence of the desired mutations can be confirmed by sequencing the nucleic acid by well known
20 methods.

The following non-limiting methods can be used to modify or mutate a given nucleic acid molecule encoding a particular recombination site to provide mutated sites that can be used in the present invention:

- 25 1. By recombination of two parental DNA sequences by site-specific (e.g. attL and attR to give attP) or other (e.g. homologous) recombination mechanisms where the parental DNA segments contain one or more base alterations resulting in the final mutated nucleic acid molecule;
2. By mutation or mutagenesis (site-specific, PCR, random, spontaneous, etc) directly of the desired nucleic acid molecule;
- 30 3. By mutagenesis (site-specific, PCR, random, spontaneous, etc) of parental DNA sequences, which are recombined to generate a desired nucleic acid molecule;

4. By reverse transcription of an RNA encoding the desired core sequence;
and

5. By *de novo* synthesis (chemical synthesis) of a sequence having the desired
base changes, or random base changes followed by sequencing or
functional analysis according to methods that are routine in the art.

The functionality of the mutant recombination sites can be demonstrated in
ways that depend on the particular characteristic that is desired. For example, the
lack of translation stop codons in a recombination site can be demonstrated by
expressing the appropriate fusion proteins. Specificity of recombination between
homologous partners can be demonstrated by introducing the appropriate
molecules into *in vitro* reactions, and assaying for recombination products as
described herein or known in the art. Other desired mutations in recombination
sites might include the presence or absence of restriction sites, translation or
transcription start signals, protein binding sites, particular coding sequences, and
other known functionalities of nucleic acid base sequences. Genetic selection
schemes for particular functional attributes in the recombination sites can be used
according to known method steps. For example, the modification of sites to
provide (from a pair of sites that do not interact) partners that do interact could
be achieved by requiring deletion, via recombination between the sites, of a DNA
sequence encoding a toxic substance. Similarly, selection for sites that remove
translation stop sequences, the presence or absence of protein binding sites, etc.,
can be easily devised by those skilled in the art.

Accordingly, the present invention also provides a nucleic acid molecule,
comprising at least one DNA segment having at least one, and preferably at least
two, engineered recombination site nucleotide sequences of the invention flanking
a selectable marker and/or a desired DNA segment, wherein at least one of said
recombination site nucleotide sequences has at least one engineered mutation that
enhances recombination *in vitro* in the formation of a Cointegrate DNA or a
Product DNA. Such engineered mutations may be in the core sequence of the
recombination site nucleotide sequence of the invention; *see* U.S. Application Nos.
08/486,139, filed June 7, 1995, 08/663,002, filed June 7, 1996 (now U.S. Patent
No. 5,888,732), 09/005,476, filed January 12, 1998, and 09/177,387, filed

October 23, 1998, the disclosures of which are all incorporated herein by reference in their entireties.

While in the preferred embodiment the recombination sites differ in sequence and do not interact with each other, it is recognized that sites comprising the same sequence, which may interact with each other, can be manipulated or engineered to inhibit recombination with each other. Such conceptions are considered and incorporated herein. For example, a protein binding site (*e.g.*, an antibody-binding site, a histone-binding site, an enzyme-binding site, or a binding site for any nucleic acid molecule-binding protein) can be engineered adjacent to one of the sites. In the presence of the protein that recognizes the engineered site, the recombinase fails to access the site and another recombination site in the nucleic acid molecule is therefore used preferentially. In the cointegrate this site can no longer react since it has been changed, *e.g.*, from attB, to attL. During or upon resolution of the cointegrate, the protein can be inactivated (*e.g.*, by antibody, heat or a change of buffer) and the second site can undergo recombination.

The nucleic acid molecules of the invention can have at least one mutation that confers at least one enhancement of said recombination, said enhancement selected from the group consisting of substantially (i) favoring integration; (ii) favoring recombination; (ii) relieving the requirement for host factors; (iii) increasing the efficiency of said Cointegrate DNA or Product DNA formation; (iv) increasing the specificity of said Cointegrate DNA or Product DNA formation; and (v) adding or deleting protein binding sites.

In other embodiments, the nucleic acid molecules of the invention may be PCR primer molecules, which comprise one or more of the recombination site sequences described herein or portions thereof, particularly those shown in Figure 9 (or sequences complementary to those shown in Figure 9), or mutants, fragments, variants or derivatives thereof, attached at the 3' end to a target-specific template sequence which specifically interacts with a target nucleic acid molecule which is to be amplified. Primer molecules according to this aspect of the invention may further comprise one or more, (*e.g.*, 1, 2, 3, 4, 5, 10, 20, 25, 50, 100, 500, 1000, or more) additional bases at their 5' ends, and preferably comprise one or more (particularly four or five) additional bases, which are preferably

guanines, at their 5' ends, to increase the efficiency of the amplification products incorporating the primer molecules in the recombinational cloning system of the invention. Such nucleic acid molecules and primers are described in detail in the examples herein, particularly in Examples 22-25.

Certain primers of the invention may comprise one or more nucleotide deletions in the *attB1*, *attB2*, *attP1*, *attP2*, *attL1*, *attL2*, *attR1* or *attR2* sequences as set forth in Figure 9. In one such aspect, for example, *attB2* primers may be constructed in which one or more of the first four nucleotides at the 5' end of the *attB2* sequence shown in Figure 9 have been deleted. Primers according to this aspect of the invention may therefore have the sequence:

(*attB2*(-1)): CCCAGCTTTCTTGTACAAAGTGGTnnnnnnnnnnnnnn . . . n

(*attB2*(-2)): CCAGCTTTCTTGTACAAAGTGGTnnnnnnnnnnnnnn . . . n

(*attB2*(-3)): CAGCTTTCTTGTACAAAGTGGTnnnnnnnnnnnnnn . . . n

(*attB2*(-4)): AGCTTTCTTGTACAAAGTGGTnnnnnnnnnnnnnn . . . n,

wherein "nnnnnnnnnnnn . . . n" at the 3' end of the primer represents a target-specific sequence of any length, for example from one base up to all of the bases of a target nucleic acid molecule (*e.g.*, a gene) or a portion thereof, the sequence and length which will depend upon the identity of the target nucleic acid molecule which is to be amplified.

The primer nucleic acid molecules according to this aspect of the invention may be produced synthetically by attaching the recombination site sequences depicted in Figure 9, or portions thereof, to the 5' end of a standard PCR target-specific primer according to methods that are well-known in the art. Alternatively, additional primer nucleic acid molecules of the invention may be produced synthetically by adding one or more nucleotide bases, which preferably correspond to one or more, preferably five or more, and more preferably six or more, contiguous nucleotides of the *att* nucleotide sequences described herein (*see, e.g.*, Example 20 herein; *see also* U.S. Application Nos. 08/663,002, filed June 7, 1996 (now U.S. Patent No. 5,888,732), 09/005,476, filed January 12, 1998, and 09/177,387, filed October 23, 1998, the disclosures of which are all incorporated herein by reference in their entireties), to the 5' end of a standard PCR target-specific primer according to methods that are well-known in the art, to provide

primers having the specific nucleotide sequences described herein. As noted above, primer nucleic acid molecules according to this aspect of the invention may also optionally comprise one, two, three, four, five, or more additional nucleotide bases at their 5' ends, and preferably will comprise four or five guanines at their 5' ends. In one particularly preferred such aspect, the primer nucleic acid molecules of the invention may comprise one or more, preferably five or more, more preferably six or more, still more preferably 6-18 or 6-25, and most preferably 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25, contiguous nucleotides or bp of the *attB1* or *attB2* nucleotide sequences depicted in Figure 9 (or nucleotides complementary thereto), linked to the 5' end of a target-specific (e.g., a gene-specific) primer molecule. Primer nucleic acid molecules according to this aspect of the invention include, but are not limited to, *attB1*- and *attB2*-derived primer nucleic acid molecules having the following nucleotide sequences:

ACAAGTTTGTACAAAAAAGCAGGCT-nnnnnnnnnnnnn . . . n
ACCACTTTGTACAAGAAAGCTGGGT-nnnnnnnnnnnnn . . . n
TGTACAAAAAAGCAGGCT-nnnnnnnnnnnnn . . . n
TGTACAAGAAAGCTGGGT-nnnnnnnnnnnnn . . . n
ACAAAAAAGCAGGCT-nnnnnnnnnnnnn . . . n
ACAAGAAAGCTGGGT-nnnnnnnnnnnnn . . . n
AAAAAGCAGGCT-nnnnnnnnnnnnn . . . n
AGAAAGCTGGGT-nnnnnnnnnnnnn . . . n
AAAAGCAGGCT-nnnnnnnnnnnnn . . . n
GAAAGCTGGGT-nnnnnnnnnnnnn . . . n
AAAGCAGGCT-nnnnnnnnnnnnn . . . n
AAAGCTGGGT-nnnnnnnnnnnnn . . . n
AAGCAGGCT-nnnnnnnnnnnnn . . . n
AAGCTGGGT-nnnnnnnnnnnnn . . . n
AGCAGGCT-nnnnnnnnnnnnn . . . n
AGCTGGGT-nnnnnnnnnnnnn . . . n
GCAGGCT-nnnnnnnnnnnnn . . . n
GCTGGGT-nnnnnnnnnnnnn . . . n

CAGGCT-nnnnnnnnnnnnn . . . n

CTGGGT-nnnnnnnnnnnnn . . . n,

wherein "nnnnnnnnnnnn . . . n" at the 3' end of the primer represents a target-specific sequence of any length, for example from one base up to all of the bases of a target nucleic acid molecule (*e.g.*, a gene) or a portion thereof, the sequence and length which will depend upon the identity of the target nucleic acid molecule which is to be amplified.

Of course, it will be apparent to one of ordinary skill from the teachings contained herein that additional primer nucleic acid molecules analogous to those specifically described herein may be produced using one or more, preferably five or more, more preferably six or more, still more preferably ten or more, 15 or more, 20 or more, 25 or more, 30 or more, etc. (through to and including all) of the contiguous nucleotides or bp of the *attP1*, *attP2*, *attL1*, *attL2*, *attR1* or *attR2* nucleotide sequences depicted in Figure 9 (or nucleotides complementary thereto), linked to the 5' end of a target-specific (*e.g.*, a gene-specific) primer molecule. As noted above, such primer nucleic acid molecules may optionally further comprise one, two, three, four, five, or more additional nucleotide bases at their 5' ends, and preferably will comprise four guanines at their 5' ends. Other primer molecules comprising the *attB1*, *attB2*, *attP1*, *attP2*, *attL1*, *attL2*, *attR1* and *attR2* sequences depicted in Figure 9, or portions thereof, may be made by one of ordinary skill without resorting to undue experimentation in accordance with the guidance provided herein.

The primers of the invention described herein are useful in producing PCR fragments having a nucleic acid molecule of interest flanked at each end by a recombination site sequence (as described in detail below in Example 9), for use in cloning of PCR-amplified DNA fragments using the recombination system of the invention (as described in detail below in Examples 8, 19 and 21-25).

Vectors

The invention also relates to vectors comprising one or more of the nucleic acid molecules of the invention, as described herein. In accordance with the invention, any vector may be used to construct the vectors of the invention. In

particular, vectors known in the art and those commercially available (and variants or derivatives thereof) may in accordance with the invention be engineered to include one or more nucleic acid molecules encoding one or more recombination sites (or portions thereof), or mutants, fragments, or derivatives thereof, for use in the methods of the invention. Such vectors may be obtained from, for example, Vector Laboratories Inc., InVitrogen, Promega, Novagen, New England Biolabs, Clontech, Roche, Pharmacia, EpiCenter, OriGenes Technologies Inc., Stratagene, Perkin Elmer, Pharmingen, Life Technologies, Inc., and Research Genetics. Such vectors may then for example be used for cloning or subcloning nucleic acid molecules of interest. General classes of vectors of particular interest include prokaryotic and/or eukaryotic cloning vectors, Expression Vectors, fusion vectors, two-hybrid or reverse two-hybrid vectors, shuttle vectors for use in different hosts, mutagenesis vectors, transcription vectors, vectors for receiving large inserts and the like.

Other vectors of interest include viral origin vectors (M13 vectors, bacterial phage λ vectors, bacteriophage P1 vectors, adenovirus vectors, herpesvirus vectors, retrovirus vectors, phage display vectors, combinatorial library vectors), high, low, and adjustable copy number vectors, vectors which have compatible replicons for use in combination in a single host (pACYC184 and pBR322) and eukaryotic episomal replication vectors (pCDM8).

Particular vectors of interest include prokaryotic Expression Vectors such as pCDNA II, pSL301, pSE280, pSE380, pSE420, pTrcHisA, B, and C, pRSET A, B, and C (Invitrogen, Inc.), pGEMEX-1, and pGEMEX-2 (Promega, Inc.), the pET vectors (Novagen, Inc.), pTrc99A, pKK223-3, the pGEX vectors, pEZZ18, pRIT2T, and pMC1871 (Pharmacia, Inc.), pKK233-2 and pKK388-1 (Clontech, Inc.), and pProEx-HT (Life Technologies, Inc.) and variants and derivatives thereof. Destination Vectors can also be made from eukaryotic Expression Vectors such as pFastBac, pFastBac HT, pFastBac DUAL, pSFV, and pTet-Splice (Life Technologies, Inc.), pEUK-C1, pPUR, pMAM, pMAMneo, pBI101, pBI121, pDR2, pCMVEBNA, and pYACneo (Clontech), pSVK3, pSVL, pMSG, pCH110, and pKK232-8 (Pharmacia, Inc.), p3'SS, pXT1, pSG5, pPbac, pMbac, pMC1neo, and pOG44 (Stratagene, Inc.), and pYES2, pAC360, pBlueBacHis A,

B, and C, pVL1392, pBsueBacIII, pCDM8, pcDNA1, pZeoSV, pcDNA3 pREP4, pCEP4, and pEBVHis (Invitrogen, Inc.) and variants or derivatives thereof.

Other vectors of particular interest include pUC18, pUC19, pBlueScript, pSPORT, cosmid, phagemids, YACs (yeast artificial chromosomes), BACs (bacterial artificial chromosomes), MACs (mammalian artificial chromosomes), pQE70, pQE60, pQE9 (Quiagen), pBS vectors, PhageScript vectors, BlueScript vectors, pNH8A, pNH16A, pNH18A, pNH46A (Stratagene), pcDNA3 (Invitrogen), pGEX, pTrsfus, pTrc99A, pET-5, pET-9, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia), pSPORT1, pSPORT2, pCMVSPORT2.0 and pSV-SPORT1 (Life Technologies, Inc.) and variants or derivatives thereof.

Additional vectors of interest include pTrxFus, pThioHis, pLEX, pTrcHis, pTrcHis2, pRSET, pBlueBacHis2, pcDNA3.1/His, pcDNA3.1(-)/Myc-His, pSecTag, pEBVHis, pPIC9K, pPIC3.5K, pAO815, pPICZ, pPICZ α , pGAPZ, pGAPZ α , pBlueBac4.5, pBlueBacHis2, pMelBac, pSinRep5, pSinHis, pIND, pIND(SP1), pVgRXR, pcDNA2.1, pYES2, pZER01.1, pZER0-2.1, pCR-Blunt, pSE280, pSE380, pSE420, pVL1392, pVL1393, pCDM8, pcDNA1.1, pcDNA1.1/Amp, pcDNA3.1, pcDNA3.1/Zeo, pSe,SV2, pRc/CMV2, pRc/RSV, pREP4, pREP7, pREP8, pREP9, pREP10, pCEP4, pEBVHis, pCR3.1, pCR2.1, pCR3.1-Uni, and pCRBac from Invitrogen; λ ExCell, λ gt11, pTrc99A, pKK223-3, pGEX-1 λ T, pGEX-2T, pGEX-2TK, pGEX-4T-1, pGEX-4T-2, pGEX-4T-3, pGEX-3X, pGEX-5X-1, pGEX-5X-2, pGEX-5X-3, pEZZ18, pRIT2T, pMC1871, pSVK3, pSVL, pMSG, pCH110, pKK232-8, pSL1180, pNEO, and pUC4K from Pharmacia; pSCREEN-1b(+), pT7Blue(R), pT7Blue-2, pCITE-4abc(+), pOCUS-2, pTAg, pET-32 LIC, pET-30 LIC, pBAC-2cp LIC, pBACgus-2cp LIC, pT7Blue-2 LIC, pT7Blue-2, λ SCREEN-1, λ BlueSTAR, pET-3abcd, pET-7abc, pET9abcd, pET11abcd, pET12abc, pET-14b, pET-15b, pET-16b, pET-17b-pET-17xb, pET-19b, pET-20b(+), pET-21abcd(+), pET-22b(+), pET-23abcd(+), pET-24abcd(+), pET-25b(+), pET-26b(+), pET-27b(+), pET-28abc(+), pET-29abc(+), pET-30abc(+), pET-31b(+), pET-32abc(+), pET-33b(+), pBAC-1, pBACgus-1, pBAC4x-1, pBACgus4x-1, pBAC-3cp, pBACgus-2cp, pBACsurf-1, plg, Signal plg, pYX, Selecta Vecta-Neo, Selecta Vecta - Hyg, and Selecta Vecta - Gpt from Novagen; pLexA, pB42AD, pGBT9, pAS2-1,

pGAD424, pACT2, pGAD GL, pGAD GH, pGAD10, pGilda, pEZM3, pEGFP, pEGFP-1, pEGFP-N, pEGFP-C, pEBFP, pGFPuv, pGFP, p6xHis-GFP, pSEAP2-Basic, pSEAP2-Contral, pSEAP2-Promoter, pSEAP2-Enhancer, p β gal-Basic, p β gal-Control, p β gal-Promoter, p β gal-Enhancer, pCMV β , pTet-Off, pTet-On, pTK-Hyg, pRetro-Off, pRetro-On, pIRES1neo, pIRES1hyg, pLXSN, pLNCX, pLAPSN, pMAMneo, pMAMneo-CAT, pMAMneo-LUC, pPUR, pSV2neo, pYEX4T-1/2/3, pYEX-S1, pBacPAK-His, pBacPAK8/9, pAcUW31, BacPAK6, pTriplEx, λ gt10, λ gt11, pWE15, and λ TriplEx from Clontech; Lambda ZAP II, pBK-CMV, pBK-RSV, pBluescript II KS +/-, pBluescript II SK +/-, pAD-GAL4, pBD-GAL4 Cam, pSurfscrip, Lambda FIX II, Lambda DASH, Lambda EMBL3, Lambda EMBL4, SuperCos, pCR-Script Amp, pCR-Script Cam, pCR-Script Direct, pBS +/-, pBC KS +/-, pBC SK +/-, Phagescript, pCAL-n-EK, pCAL-n, pCAL-c, pCAL-kc, pET-3abcd, pET-11abcd, pSPUTK, pESP-1, pCMVLacI, pOPRSVI/MCS, pOPI3 CAT, pXT1, pSG5, pPbac, pMbac, pMC1neo, pMC1neo Poly A, pOG44, pOG45, pFRT β GAL, pNEO β GAL, pRS403, pRS404, pRS405, pRS406, pRS413, pRS414, pRS415, and pRS416 from Stratagene.

Two-hybrid and reverse two-hybrid vectors of particular interest include pPC86, pDBLeu, pDBTrp, pPC97, p2.5, pGAD1-3, pGAD10, pAct, pACT2, pGADGL, pGADGH, pAS2-1, pGAD424, pGBT8, pGBT9, pGAD-GAL4, pLexA, pBD-GAL4, pHISi, pHISi-1, placZi, pB42AD, pDG202, pJK202, pJG4-5, pNLexA, pYESTrp and variants or derivatives thereof.

Yeast Expression Vectors of particular interest include pESP-1, pESP-2, pESC-His, pESC-Trp, pESC-URA, pESC-Leu (Stratagene), pRS401, pRS402, pRS411, pRS412, pRS421, pRS422, and variants or derivatives thereof.

According to the invention, the vectors comprising one or more nucleic acid molecules encoding one or more recombination sites, or mutants, variants, fragments, or derivatives thereof, may be produced by one of ordinary skill in the art without resorting to undue experimentation using standard molecular biology methods. For example, the vectors of the invention may be produced by introducing one or more of the nucleic acid molecules encoding one or more recombination sites (or mutants, fragments, variants or derivatives thereof) into one or more of the vectors described herein, according to the methods described,

for example, in Maniatis *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (1982). In a related aspect of the invention, the vectors may be engineered to contain, in addition to one or more nucleic acid molecules encoding one or more recombination sites (or portions thereof), one or more additional physical or functional nucleotide sequences, such as those encoding one or more multiple cloning sites, one or more transcription termination sites, one or more transcriptional regulatory sequences (e.g., one or more promoters, enhancers, or repressors), one or more selection markers or modules, one or more genes or portions of genes encoding a protein or polypeptide of interest, one or more translational signal sequences, one or more nucleotide sequences encoding a fusion partner protein or peptide (e.g., GST, His₆, or thioredoxin), one or more origins of replication, and one or more 5' or 3' polynucleotide tails (particularly a poly-G tail). According to this aspect of the invention, the one or more recombination site nucleotide sequences (or portions thereof) may optionally be operably linked to the one or more additional physical or functional nucleotide sequences described herein.

Preferred vectors according to this aspect of the invention include, but are not limited to: pENTR1A (Figures 10A and 10B), pENTR2B (Figures 11A and 11B), pENTR3C (Figures 12A and 12B), pENTR4 (Figures 13A and 13B), pENTR5 (Figures 14A and 14B), pENTR6 (Figures 15A and 15B), pENTR7 (Figures 16A and 16B), pENTR8 (Figures 17A and 17B), pENTR9 (Figures 18A and 18B), pENTR10 (Figures 19A and 19B), pENTR11 (Figures 20A and 20B), pDEST1 (Figures 21A-D), pDEST2 (Figure 22A-D), pDEST3 (Figure 23A-D), pDEST4 (Figure 24A-D), pDEST5 (Figure 25A-D), pDEST6 (Figure 26A-D), pDEST7 (Figure 27A-C), pDEST8 (Figure 28A-D), pDEST9 (Figure 29A-E), pDEST10 (Figure 30A-D), pDEST11 (Figure 31A-D), pDEST12.2 (also known as pDEST12) (Figure 32A-D), pDEST13 (Figure 33A-C), pDEST14 (Figure 34A-D), pDEST15 (Figure 35A-D), pDEST16 (Figure 36A-D), pDEST17 (Figure 37A-D), pDEST18 (Figure 38A-D), pDEST19 (Figure 39A-D), pDEST20 (Figure 40A-D), pDEST21 (Figure 41A-E), pDEST22 (Figure 42A-D), pDEST23 (Figure 43A-D), pDEST24 (Figure 44A-D), pDEST25 (Figure 45A-D), pDEST26 (Figure 46A-D), pDEST27 (Figure 47A-D), pEXP501 (also known

as pCMVSPORT6) (Figure 48A-B), pDONR201 (also known as pENTR21 attP vector or pAttPkan Donor Vector) (Figure 49), pDONR202 (Figure 50), pDONR203 (also known as pEZ15812) (Figure 51), pDONR204 (Figure 52), pDONR205 (Figure 53), pDONR206 (also known as pENTR22 attP vector or pAttPgen Donor Vector) (Figure 54), pMAB58 (Figure 87), pMAB62 (Figure 88), pDEST28 (Figure 90), pDEST29 (Figure 91), pDEST30 (Figure 92), pDEST31 (Figure 93), pDEST32 (Figure 94), pDEST33 (Figure 95), pDEST34 (Figure 96), pDONR207 (Figure 97), pMAB85 (Figure 98), pMAB86 (Figure 99), and fragments, mutants, variants, and derivatives thereof. However, it will be understood by one of ordinary skill that the present invention also encompasses other vectors not specifically designated herein, which comprise one or more of the isolated nucleic acid molecules of the invention encoding one or more recombination sites or portions thereof (or mutants, fragments, variants or derivatives thereof), and which may further comprise one or more additional physical or functional nucleotide sequences described herein which may optionally be operably linked to the one or more nucleic acid molecules encoding one or more recombination sites or portions thereof. Such additional vectors may be produced by one of ordinary skill according to the guidance provided in the present specification.

Polymerases

Preferred polypeptides having reverse transcriptase activity (*i.e.*, those polypeptides able to catalyze the synthesis of a DNA molecule from an RNA template) for use in accordance with the present invention include, but are not limited to Moloney Murine Leukemia Virus (M-MLV) reverse transcriptase, Rous Sarcoma Virus (RSV) reverse transcriptase, Avian Myeloblastosis Virus (AMV) reverse transcriptase, Rous Associated Virus (RAV) reverse transcriptase, Myeloblastosis Associated Virus (MAV) reverse transcriptase, Human Immunodeficiency Virus (HIV) reverse transcriptase, retroviral reverse transcriptase, retrotransposon reverse transcriptase, hepatitis B reverse transcriptase, cauliflower mosaic virus reverse transcriptase and bacterial reverse transcriptase. Particularly preferred are those polypeptides having reverse

transcriptase activity that are also substantially reduced in RNase H activity (*i.e.*, “RNase H” polypeptides). By a polypeptide that is “substantially reduced in RNase H activity” is meant that the polypeptide has less than about 20%, more preferably less than about 15%, 10% or 5%, and most preferably less than about 2%, of the RNase H activity of a wildtype or RNase H⁺ enzyme such as wildtype M-MLV reverse transcriptase. The RNase H activity may be determined by a variety of assays, such as those described, for example, in U.S. Patent No. 5,244,797, in Kotewicz, M.L. *et al.*, *Nucl. Acids Res.* 16:265 (1988) and in Gerard, G.F., *et al.*, *FOCUS* 14(5):91 (1992), the disclosures of all of which are fully incorporated herein by reference. Suitable RNase H⁺ polypeptides for use in the present invention include, but are not limited to, M-MLV H⁺ reverse transcriptase, RSV H⁺ reverse transcriptase, AMV H⁺ reverse transcriptase, RAV H⁺ reverse transcriptase, MAV H⁺ reverse transcriptase, HIV H⁺ reverse transcriptase, THERMOSCRIPT™ reverse transcriptase and THERMOSCRIPT™ II reverse transcriptase, and SUPERScript™ I reverse transcriptase and SUPERScript™ II reverse transcriptase, which are obtainable, for example, from Life Technologies, Inc. (Rockville, Maryland). See generally published PCT application WO 98/47912.

Other polypeptides having nucleic acid polymerase activity suitable for use in the present methods include thermophilic DNA polymerases such as DNA polymerase I, DNA polymerase III, Klenow fragment, T7 polymerase, and T5 polymerase, and thermostable DNA polymerases including, but not limited to, *Thermus thermophilus* (*Tth*) DNA polymerase, *Thermus aquaticus* (*Taq*) DNA polymerase, *Thermotoga neopolitana* (*Tne*) DNA polymerase, *Thermotoga maritima* (*Tma*) DNA polymerase, *Thermococcus litoralis* (*Tli* or VENT®) DNA polymerase, *Pyrococcus furiosus* (*Pfu*) DNA polymerase, *Pyrococcus* species GB-D (or DEEPVENT®) DNA polymerase, *Pyrococcus woosii* (*Pwo*) DNA polymerase, *Bacillus stearothermophilus* (*Bst*) DNA polymerase, *Sulfolobus acidocaldarius* (*Sac*) DNA polymerase, *Thermoplasma acidophilum* (*Tac*) DNA polymerase, *Thermus flavus* (*Tfl/Tub*) DNA polymerase, *Thermus ruber* (*Tru*) DNA polymerase, *Thermus brockianus* (DYNAZYME®) DNA polymerase, *Methanobacterium thermoautotrophicum* (*Mth*) DNA polymerase, and mutants,

variants and derivatives thereof. Such polypeptides are available commercially, for example from Life Technologies, Inc. (Rockville, MD), New England BioLabs (Beverly, MA), and Sigma/Aldrich (St. Louis, MO).

Host Cells

The invention also relates to host cells comprising one or more of the nucleic acid molecules or vectors of the invention, particularly those nucleic acid molecules and vectors described in detail herein. Representative host cells that may be used according to this aspect of the invention include, but are not limited to, bacterial cells, yeast cells, plant cells and animal cells. Preferred bacterial host cells include *Escherichia* spp. cells (particularly *E. coli* cells and most particularly *E. coli* strains DH10B, Stbl2, DH5 α , DB3, DB3.1 (preferably *E. coli* LIBRARY EFFICIENCY \circledR DB3.1TM Competent Cells; Life Technologies, Inc., Rockville, MD), DB4 and DB5; see U.S. Provisional Application No. 60/122,392, filed on March 2, 1999, the disclosure of which is incorporated by reference herein in its entirety), *Bacillus* spp. cells (particularly *B. subtilis* and *B. megaterium* cells), *Streptomyces* spp. cells, *Erwinia* spp. cells, *Klebsiella* spp. cells, *Serratia* spp. cells (particularly *S. marcessans* cells), *Pseudomonas* spp. cells (particularly *P. aeruginosa* cells), and *Salmonella* spp. cells (particularly *S. typhimurium* and *S. typhi* cells). Preferred animal host cells include insect cells (most particularly *Drosophila melanogaster* cells, *Spodoptera frugiperda* Sf9 and Sf21 cells and *Trichoplusia* High-Five cells), nematode cells (particularly *C. elegans* cells), avian cells, amphibian cells (particularly *Xenopus laevis* cells), reptilian cells, and mammalian cells (most particularly CHO, COS, VERO, BHK and human cells). Preferred yeast host cells include *Saccharomyces cerevisiae* cells and *Pichia pastoris* cells. These and other suitable host cells are available commercially, for example from Life Technologies, Inc. (Rockville, Maryland), American Type Culture Collection (Manassas, Virginia), and Agricultural Research Culture Collection (NRRL; Peoria, Illinois).

Methods for introducing the nucleic acid molecules and/or vectors of the invention into the host cells described herein, to produce host cells comprising one or more of the nucleic acid molecules and/or vectors of the invention, will be

familiar to those of ordinary skill in the art. For instance, the nucleic acid molecules and/or vectors of the invention may be introduced into host cells using well known techniques of infection, transduction, transfection, and transformation. The nucleic acid molecules and/or vectors of the invention may be introduced alone or in conjunction with other the nucleic acid molecules and/or vectors. Alternatively, the nucleic acid molecules and/or vectors of the invention may be introduced into host cells as a precipitate, such as a calcium phosphate precipitate, or in a complex with a lipid. Electroporation also may be used to introduce the nucleic acid molecules and/or vectors of the invention into a host. Likewise, such molecules may be introduced into chemically competent cells such as *E. coli*. If the vector is a virus, it may be packaged *in vitro* or introduced into a packaging cell and the packaged virus may be transduced into cells. Hence, a wide variety of techniques suitable for introducing the nucleic acid molecules and/or vectors of the invention into cells in accordance with this aspect of the invention are well known and routine to those of skill in the art. Such techniques are reviewed at length, for example, in Sambrook, J., *et al.*, *Molecular Cloning, a Laboratory Manual*, 2nd Ed., Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, pp. 16.30-16.55 (1989), Watson, J.D., *et al.*, *Recombinant DNA*, 2nd Ed., New York: W.H. Freeman and Co., pp. 213-234 (1992), and Winnacker, E.-L., *From Genes to Clones*, New York: VCH Publishers (1987), which are illustrative of the many laboratory manuals that detail these techniques and which are incorporated by reference herein in their entireties for their relevant disclosures.

Polypeptides

In another aspect, the invention relates to polypeptides encoded by the nucleic acid molecules of the invention (including polypeptides and amino acid sequences encoded by all possible reading frames of the nucleic acid molecules of the invention), and to methods of producing such polypeptides. Polypeptides of the present invention include purified or isolated natural products, products of chemical synthetic procedures, and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, insect, mammalian, avian and higher plant cells.

The polypeptides of the invention may be produced by synthetic organic chemistry, and are preferably produced by standard recombinant methods, employing one or more of the host cells of the invention comprising the vectors or isolated nucleic acid molecules of the invention. According to the invention, polypeptides are produced by cultivating the host cells of the invention (which comprise one or more of the nucleic acid molecules of the invention, preferably contained within an Expression Vector) under conditions favoring the expression of the nucleotide sequence contained on the nucleic acid molecule of the invention, such that the polypeptide encoded by the nucleic acid molecule of the invention is produced by the host cell. As used herein, "conditions favoring the expression of the nucleotide sequence" or "conditions favoring the production of a polypeptide" include optimal physical (*e.g.*, temperature, humidity, etc.) and nutritional (*e.g.*, culture medium, ionic) conditions required for production of a recombinant polypeptide by a given host cell. Such optimal conditions for a variety of host cells, including prokaryotic (bacterial), mammalian, insect, yeast, and plant cells will be familiar to one of ordinary skill in the art, and may be found, for example, in Sambrook, J., *et al.*, *Molecular Cloning, A Laboratory Manual, 2nd Ed.*, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, (1989), Watson, J.D., *et al.*, *Recombinant DNA, 2nd Ed.*, New York: W.H. Freeman and Co., and Winnacker, E.-L., *From Genes to Clones*, New York: VCH Publishers (1987).

In some aspects, it may be desirable to isolate or purify the polypeptides of the invention (*e.g.*, for production of antibodies as described below), resulting in the production of the polypeptides of the invention in isolated form. The polypeptides of the invention can be recovered and purified from recombinant cell cultures by well-known methods of protein purification that are routine in the art, including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. For example, His6 or GST fusion tags on polypeptides made by the methods of the invention may be isolated using appropriate affinity chromatography matrices which bind polypeptides bearing

His6 or GST tags, as will be familiar to one of ordinary skill in the art. Polypeptides of the present invention include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes.

Isolated polypeptides of the invention include those comprising the amino acid sequences encoded by one or more of the reading frames of the polynucleotides comprising one or more of the recombination site-encoding nucleic acid molecules of the invention, including those encoding *attB1*, *attB2*, *attP1*, *attP2*, *attL1*, *attL2*, *attR1* and *attR2* having the nucleotide sequences set forth in Figure 9 (or nucleotide sequences complementary thereto), or fragments, variants, mutants and derivatives thereof; the complete amino acid sequences encoded by the polynucleotides contained in the deposited clones described herein; the amino acid sequences encoded by polynucleotides which hybridize under stringent hybridization conditions to polynucleotides having the nucleotide sequences encoding the recombination site sequences of the invention as set forth in Figure 9 (or a nucleotide sequence complementary thereto); or a peptide or polypeptide comprising a portion or a fragment of the above polypeptides. The invention also relates to additional polypeptides having one or more additional amino acids linked (typically by peptidyl bonds to form a nascent polypeptide) to the polypeptides encoded by the recombination site nucleotide sequences or the deposited clones. Such additional amino acid residues may comprise one or more functional peptide sequences, for example one or more fusion partner peptides (*e.g.*, GST, His₆, Trx, etc.) and the like.

As used herein, the terms "protein," "peptide," "oligopeptide" and "polypeptide" are considered synonymous (as is commonly recognized) and each term can be used interchangeably as the context requires to indicate a chain of two or more amino acids, preferably five or more amino acids, or more preferably ten

or more amino acids, coupled by (a) peptidyl linkage(s), unless otherwise defined in the specific contexts below. As is commonly recognized in the art, all polypeptide formulas or sequences herein are written from left to right and in the direction from amino terminus to carboxy terminus.

5 It will be recognized by those of ordinary skill in the art that some amino acid sequences of the polypeptides of the invention can be varied without significant effect on the structure or function of the polypeptides. If such differences in sequence are contemplated, it should be remembered that there will be critical areas on the protein which determine structure and activity. In general, it is possible to replace residues which form the tertiary structure, provided that
10 residues performing a similar function are used. In other instances, the type of residue may be completely unimportant if the alteration occurs at a non-critical region of the polypeptide.

Thus, the invention further includes variants of the polypeptides of the invention, including allelic variants, which show substantial structural homology
15 to the polypeptides described herein, or which include specific regions of these polypeptides such as the portions discussed below. Such mutants may include deletions, insertions, inversions, repeats, and type substitutions (for example, substituting one hydrophilic residue for another, but not strongly hydrophilic for
20 strongly hydrophobic as a rule). Small changes or such "neutral" or "conservative" amino acid substitutions will generally have little effect on activity.

Typical conservative substitutions are the replacements, one for another, among the aliphatic amino acids Ala, Val, Leu and Ile; interchange of the hydroxylated residues Ser and Thr; exchange of the acidic residues Asp and Glu;
25 substitution between the amidated residues Asn and Gln; exchange of the basic residues Lys and Arg; and replacements among the aromatic residues Phe and Tyr.

Thus, the fragment, derivative or analog of the polypeptides of the invention, such as those comprising peptides encoded by the recombination site nucleotide sequences described herein, may be (i) one in which one or more of the amino acid
30 residues are substituted with a conservative or non-conservative amino acid residue (preferably a conservative amino acid residue), and such substituted amino acid residue may be encoded by the genetic code or may be an amino acid (e.g.,

desmosine, citrulline, ornithine, etc.) that is not encoded by the genetic code; (ii) one in which one or more of the amino acid residues includes a substituent group (e.g., a phosphate, hydroxyl, sulfate or other group) in addition to the normal "R" group of the amino acid; (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which additional amino acids are fused to the mature polypeptide, such as an immunoglobulin Fc region peptide, a leader or secretory sequence, a sequence which is employed for purification of the mature polypeptide (such as GST) or a proprotein sequence. Such fragments, derivatives and analogs are intended to be encompassed by the present invention, and are within the scope of those skilled in the art from the teachings herein and the state of the art at the time of invention.

The polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. Recombinantly produced versions of the polypeptides of the invention can be substantially purified by the one-step method described in Smith and Johnson, *Gene* 67:31-40 (1988). As used herein, the term "substantially purified" means a preparation of an individual polypeptide of the invention wherein at least 50%, preferably at least 60%, 70%, or 75% and more preferably at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% (by mass) of contaminating proteins (*i.e.*, those that are not the individual polypeptides described herein or fragments, variants, mutants or derivatives thereof) have been removed from the preparation.

The polypeptides of the present invention include those which are at least about 50% identical, at least 60% identical, at least 65% identical, more preferably at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical, to the polypeptides described herein. For example, preferred *attB1*-containing polypeptides of the invention include those that are at least about 50% identical, at least 60% identical, at least 65% identical, more preferably at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical,

to the polypeptide(s) encoded by the three reading frames of a polynucleotide comprising a nucleotide sequence of *attB1* having a nucleic acid sequence as set forth in Figure 9 (or a nucleic acid sequence complementary thereto), to a polypeptide encoded by a polynucleotide contained in the deposited cDNA clones described herein, or to a polypeptide encoded by a polynucleotide hybridizing under stringent conditions to a polynucleotide comprising a nucleotide sequence of *attB1* having a nucleic acid sequence as set forth in Figure 9 (or a nucleic acid sequence complementary thereto). Analogous polypeptides may be prepared that are at least about 65% identical, more preferably at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical, to the *attB2*, *attP1*, *attP2*, *attL1*, *attL2*, *attR1* and *attR2* polypeptides of the invention as depicted in Figure 9. The present polypeptides also include portions or fragments of the above-described polypeptides with at least 5, 10, 15, 20, or 25 amino acids.

By a polypeptide having an amino acid sequence at least, for example, 65% "identical" to a reference amino acid sequence of a given polypeptide of the invention is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to 35 amino acid alterations per each 100 amino acids of the reference amino acid sequence of a given polypeptide of the invention. In other words, to obtain a polypeptide having an amino acid sequence at least 65% identical to a reference amino acid sequence, up to 35% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 35% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino (N-) or carboxy (C-) terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence. As a practical matter, whether a given amino acid sequence is, for example, at least 65% identical to the amino acid sequence of a given polypeptide of the invention can be determined

conventionally using known computer programs such as those described above for nucleic acid sequence identity determinations, or more preferably using the CLUSTAL W program (Thompson, J.D., *et al.*, *Nucleic Acids Res.* 22:4673-4680 (1994)).

5 The polypeptides of the present invention can be used as molecular weight markers on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art. In addition, as described in detail below, the polypeptides of the present invention can be used to raise polyclonal and monoclonal antibodies which are useful in a variety of assays for detecting
10 protein expression, localization, detection of interactions with other molecules, or for the isolation of a polypeptide (including a fusion polypeptide) of the invention.

 In another aspect, the present invention provides a peptide or polypeptide comprising an epitope-bearing portion of a polypeptide of the invention, which may be used to raise antibodies, particularly monoclonal antibodies, that bind
15 specifically to a one or more of the polypeptides of the invention. The epitope of this polypeptide portion is an immunogenic or antigenic epitope of a polypeptide of the invention. An "immunogenic epitope" is defined as a part of a protein that elicits an antibody response when the whole protein is the immunogen. These immunogenic epitopes are believed to be confined to a few loci on the molecule.
20 On the other hand, a region of a protein molecule to which an antibody can bind is defined as an "antigenic epitope." The number of immunogenic epitopes of a protein generally is less than the number of antigenic epitopes (*see, e.g.*, Geysen *et al.*, *Proc. Natl. Acad. Sci. USA* 81:3998- 4002 (1983)).

 As to the selection of peptides or polypeptides bearing an antigenic epitope
25 (*i.e.*, that contain a region of a protein molecule to which an antibody can bind), it is well-known in the art that relatively short synthetic peptides that mimic part of a protein sequence are routinely capable of eliciting an antiserum that reacts with the partially mimicked protein (*see, e.g.*, Sutcliffe, J.G., *et al.*, *Science* 219:660-666 (1983)). Peptides capable of eliciting protein-reactive sera are
30 frequently represented in the primary sequence of a protein, can be characterized by a set of simple chemical rules, and are not confined to the immunodominant regions of intact proteins (*i.e.*, immunogenic epitopes) or to the amino or carboxy

termini. Peptides that are extremely hydrophobic and those of six or fewer residues generally are ineffective at inducing antibodies that bind to the mimicked protein; longer peptides, especially those containing proline residues, usually are effective (Sutcliffe, J.G., *et al.*, *Science* 219:660-666 (1983)).

5 Epitope-bearing peptides and polypeptides of the invention designed according to the above guidelines preferably contain a sequence of at least five, more preferably at least seven or more amino acids contained within the amino acid sequence of a polypeptide of the invention. However, peptides or polypeptides comprising a larger portion of an amino acid sequence of a polypeptide of the invention, containing about 30 to about 50 amino acids, or any length up to and including the entire amino acid sequence of a given polypeptide of the invention, also are considered epitope-bearing peptides or polypeptides of the invention and also are useful for inducing antibodies that react with the mimicked protein. Preferably, the amino acid sequence of the epitope-bearing peptide is selected to provide substantial solubility in aqueous solvents (*i.e.*, the sequence includes relatively hydrophilic residues and highly hydrophobic sequences are preferably avoided); sequences containing proline residues are particularly preferred.

15 Non-limiting examples of epitope-bearing polypeptides or peptides that can be used to generate antibodies specific for the polypeptides of the invention include certain epitope-bearing regions of the polypeptides comprising amino acid sequences encoded by polynucleotides comprising one or more of the recombination site-encoding nucleic acid molecules of the invention, including those encoding *attB1*, *attB2*, *attP1*, *attP2*, *attL1*, *attL2*, *attR1* and *attR2* having the nucleotide sequences set forth in Figure 9 (or a nucleotide sequence complementary thereto); the complete amino acid sequences encoded by the three reading frames of the polynucleotides contained in the deposited clones described herein; and the amino acid sequences encoded by all reading frames of polynucleotides which hybridize under stringent hybridization conditions to polynucleotides having the nucleotide sequences encoding the recombination site sequences (or portions thereof) of the invention as set forth in Figure 9 (or a nucleic acid sequence complementary thereto). Other epitope-bearing polypeptides or peptides that may be used to generate antibodies specific for the polypeptides

of the invention will be apparent to one of ordinary skill in the art based on the primary amino acid sequences of the polypeptides of the invention described herein, via the construction of Kyte-Doolittle hydrophilicity and Jameson-Wolf antigenic index plots of the polypeptides of the invention using, for example, PROTEAN computer software (DNASTAR, Inc.; Madison, Wisconsin).

The epitope-bearing peptides and polypeptides of the invention may be produced by any conventional means for making peptides or polypeptides including recombinant means using nucleic acid molecules of the invention. For instance, a short epitope-bearing amino acid sequence may be fused to a larger polypeptide which acts as a carrier during recombinant production and purification, as well as during immunization to produce anti-peptide antibodies. Epitope-bearing peptides also may be synthesized using known methods of chemical synthesis (*see, e.g.*, U.S. Patent No. 4,631,211 and Houghten, R. A., *Proc. Natl. Acad. Sci. USA* 82:5131-5135 (1985), both of which are incorporated by reference herein in their entireties).

As one of skill in the art will appreciate, the polypeptides of the present invention and epitope-bearing fragments thereof may be immobilized onto a solid support, by techniques that are well-known and routine in the art. By "solid support" is intended any solid support to which a peptide can be immobilized. Such solid supports include, but are not limited to nitrocellulose, diazocellulose, glass, polystyrene, polyvinylchloride, polypropylene, polyethylene, dextran, Sepharose, agar, starch, nylon, beads and microtitre plates. Linkage of the peptide of the invention to a solid support can be accomplished by attaching one or both ends of the peptide to the support. Attachment may also be made at one or more internal sites in the peptide. Multiple attachments (both internal and at the ends of the peptide) may also be used according to the invention. Attachment can be via an amino acid linkage group such as a primary amino group, a carboxyl group, or a sulfhydryl (SH) group or by chemical linkage groups such as with cyanogen bromide (CNBr) linkage through a spacer. For non-covalent attachments to the support, addition of an affinity tag sequence to the peptide can be used such as GST (Smith, D.B., and Johnson, K.S., *Gene* 67:31 (1988)), polyhistidines (Hochuli, E., *et al.*, *J. Chromatog.* 411:77 (1987)), or biotin. Such affinity tags

may be used for the reversible attachment of the peptide to the support. Such immobilized polypeptides or fragments may be useful, for example, in isolating antibodies directed against one or more of the polypeptides of the invention, or other proteins or peptides that recognize other proteins or peptides that bind to one or more of the polypeptides of the invention, as described below.

As one of skill in the art will also appreciate, the polypeptides of the present invention and the epitope-bearing fragments thereof described herein can be combined with one or more fusion partner proteins or peptides, or portions thereof, including but not limited to GST, His₆, Trx, and portions of the constant domain of immunoglobulins (Ig), resulting in chimeric or fusion polypeptides. These fusion polypeptides facilitate purification of the polypeptides of the invention (EP 0 394 827; Traunecker *et al.*, *Nature* 331:84-86 (1988)) for use in analytical or diagnostic (including high-throughput) format.

Antibodies

In another aspect, the invention relates to antibodies that recognize and bind to the polypeptides (or epitope-bearing fragments thereof) or nucleic acid molecules (or portions thereof) of the invention. In a related aspect, the invention relates to antibodies that recognize and bind to one or more polypeptides encoded by all reading frames of one or more recombination site nucleic acid sequences or portions thereof, or to one or more nucleic acid molecules comprising one or more recombination site nucleic acid sequences or portions thereof, including but not limited to *att* sites (including *attB1*, *attB2*, *attP1*, *attP2*, *attL1*, *attL2*, *attR1*, *attR2* and the like), *lox* sites (*e.g.*, *loxP*, *loxP511*, and the like), FRT, and the like, or mutants, fragments, variants and derivatives thereof. See generally U.S. Patent No. 5,888,732, which is incorporated herein by reference in its entirety. The antibodies of the present invention may be polyclonal or monoclonal, and may be prepared by any of a variety of methods and in a variety of species according to methods that are well-known in the art. See, for instance, U.S. Patent No. 5,587,287; Sutcliffe, J.G., *et al.*, *Science* 219:660-666 (1983); Wilson *et al.*, *Cell* 37: 767 (1984); and Bittle, F.J., *et al.*, *J. Gen. Virol.* 66:2347-2354 (1985). Antibodies specific for any of the polypeptides or nucleic acid molecules described

herein, such as antibodies specifically binding to one or more of the polypeptides encoded by the recombination site nucleotide sequences, or one or more nucleic acid molecules, described herein or contained in the deposited clones, antibodies against fusion polypeptides (*e.g.*, binding to fusion polypeptides between one or more of the fusion partner proteins and one or more of the recombination site polypeptides of the invention, as described herein), and the like, can be raised against the intact polypeptides or polynucleotides of the invention or one or more antigenic polypeptide fragments thereof.

As used herein, the term "antibody" (Ab) may be used interchangeably with the terms "polyclonal antibody" or "monoclonal antibody" (mAb), except in specific contexts as described below. These terms, as used herein, are meant to include intact molecules as well as antibody fragments (such as, for example, Fab and F(ab')₂ fragments) which are capable of specifically binding to a polypeptide or nucleic acid molecule of the invention or a portion thereof. It will therefore be appreciated that, in addition to the intact antibodies of the invention, Fab, F(ab')₂ and other fragments of the antibodies described herein, and other peptides and peptide fragments that bind one or more polypeptides or polynucleotides of the invention, are also encompassed within the scope of the invention. Such antibody fragments are typically produced by proteolytic cleavage of intact antibodies, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')₂ fragments). Antibody fragments, and peptides or peptide fragments, may also be produced through the application of recombinant DNA technology or through synthetic chemistry.

Epitope-bearing peptides and polypeptides, and nucleic acid molecules or portions thereof, of the invention may be used to induce antibodies according to methods well known in the art, as generally described herein (*see, e.g.*, Sutcliffe, *et al.*, *supra*; Wilson, *et al.*, *supra*; and Bittle, F. J., *et al.*, *J. Gen. Virol.* 66:2347-2354 (1985)).

Polyclonal antibodies according to this aspect of the invention may be made by immunizing an animal with one or more of the polypeptides or nucleic acid molecules of the invention described herein or portions thereof according to standard techniques (*see, e.g.*, Harlow, E., and Lane, D., *Antibodies: A*

Laboratory Manual, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press (1988); Kaufman, P.B., *et al.*, In: *Handbook of Molecular and Cellular Methods in Biology and Medicine*, Boca Raton, Florida: CRC Press, pp. 468-469 (1995)). For producing antibodies that recognize and bind to the polypeptides or nucleic acid molecules of the invention or portions thereof, animals may be immunized with free peptide or free nucleic acid molecules; however, antibody titer may be boosted by coupling of the peptide to a macromolecular carrier, such as albumin, KLH, or tetanus toxoid (particularly for producing antibodies against the nucleic acid molecules of the invention or portions thereof; *see* Harlow and Lane, *supra*, at page 154), or to a solid phase carrier such as a latex or glass microbead. For instance, peptides containing cysteine may be coupled to carrier using a linker such as m-maleimidobenzoyl-N- hydroxysuccinimide ester (MBS), while other peptides may be coupled to carrier using a more general linking agent such as glutaraldehyde. Animals such as rabbits, rats and mice may be immunized with either free (if the polypeptide immunogen is larger than about 25 amino acids in length) or carrier-coupled peptides or nucleic acid molecules, for instance, by intraperitoneal and/or intradermal injection of emulsions containing about 100 μ g peptide, polynucleotide, or carrier protein, and Freund's adjuvant. Several booster injections may be needed, for instance, at intervals of about two weeks, to provide a useful titer of antibody which can be detected, for example, by ELISA assay using free peptide or nucleic acid molecule adsorbed to a solid surface. In another approach, cells expressing one or more of the polypeptides or polynucleotides of the invention or an antigenic fragment thereof can be administered to an animal in order to induce the production of sera containing polyclonal antibodies, according to routine immunological methods. In yet another method, a preparation of one or more of the polypeptides or polynucleotides of the invention is prepared and purified as described herein, to render it substantially free of natural contaminants. Such a preparation may then be introduced into an animal in order to produce polyclonal antisera of greater specific activity. The titer of antibodies in serum from an immunized animal, regardless of the method of immunization used, may be increased by selection of anti-peptide or anti-polynucleotide antibodies, for

instance, by adsorption to the peptide or polynucleotide on a solid support and elution of the selected antibodies according to methods well known in the art.

In an alternative method, the antibodies of the present invention are monoclonal antibodies (or fragments thereof which bind to one or more of the polypeptides of the invention). Such monoclonal antibodies can be prepared using hybridoma technology (Kohler *et al.*, *Nature* 256:495 (1975); Köhler *et al.*, *Eur. J. Immunol.* 6:511 (1976); Köhler *et al.*, *Eur. J. Immunol.* 6:292 (1976); Hammerling *et al.*, In: *Monoclonal Antibodies and T-Cell Hybridomas*, Elsevier, N.Y., pp. 563-681 (1981)). In general, such procedures involve immunizing an animal (preferably a mouse) with a polypeptide or polynucleotide of the invention (or a fragment thereof), or with a cell expressing a polypeptide or polynucleotide of the invention (or a fragment thereof). The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP₂O), available from the American Type Culture Collection, Rockville, Maryland. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands *et al.* (*Gastroenterol.* 80:225-232 (1981)). The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding one or more of the polypeptides or nucleic acid molecules of the invention, or fragments thereof. Hence, the present invention also provides hybridoma cells and cell lines producing monoclonal antibodies of the invention, particularly that recognize and bind to one or more of the polypeptides or nucleic acid molecules of the invention.

Alternatively, additional antibodies capable of binding to one or more of the polypeptides of the invention, or fragments thereof, may be produced in a two-step procedure through the use of anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and that, therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, antibodies specific for one or more of the polypeptides or polynucleotides of the invention, prepared as described above, are used to immunize an animal, preferably a mouse. The splenocytes of such an

animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to an antibody specific for one or more of the polypeptides or polynucleotides of the invention can be blocked by polypeptides of the invention themselves. Such antibodies comprise anti-idiotypic antibodies to the antibodies recognizing one or more of the polypeptides or polynucleotides of the invention, and can be used to immunize an animal to induce formation of further antibodies specific for one or more of the polypeptides or polynucleotides of the invention.

For use, the antibodies of the invention may optionally be detectably labeled by covalent or non-covalent attachment of one or more labels, including but not limited to chromogenic, enzymatic, radioisotopic, isotopic, fluorescent, toxic, chemiluminescent, or nuclear magnetic resonance contrast agents or other labels.

Examples of suitable enzyme labels include malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast-alcohol dehydrogenase, alpha-glycerol phosphate dehydrogenase, triose phosphate isomerase, peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase, and acetylcholine esterase.

Examples of suitable radioisotopic labels include ^3H , ^{111}In , ^{125}I , ^{131}I , ^{32}P , ^{35}S , ^{14}C , ^{51}Cr , ^{57}Co , ^{58}Co , ^{59}Fe , ^{75}Se , ^{152}Eu , ^{90}Y , ^{67}Cu , ^{217}At , ^{212}Pb , ^{47}Sc , ^{109}Pd , etc. ^{111}In is a preferred isotope where in vivo imaging is used since it avoids the problem of dehalogenation of the ^{125}I or ^{131}I -labeled monoclonal antibody by the liver. In addition, this radionuclide has a more favorable gamma emission energy for imaging (Perkins *et al.*, *Eur. J. Nucl. Med.* 10:296-301 (1985); Carasquillo *et al.*, *J. Nucl. Med.* 28:281-287 (1987)). For example, ^{111}In coupled to monoclonal antibodies with 1-(P-isothiocyanatobenzyl)-DPTA has shown little uptake in non-tumorous tissues, particularly the liver, and therefore enhances specificity of tumor localization (Esteban *et al.*, *J. Nucl. Med.* 28:861-870 (1987)).

Examples of suitable non-radioactive isotopic labels include ^{157}Gd , ^{55}Mn , ^{162}Dy , ^{52}Tr , and ^{56}Fe .

Examples of suitable fluorescent labels include an ^{152}Eu label, a fluorescein label, an isothiocyanate label, a rhodamine label, a phycoerythrin label, a

phycocyanin label, an allophycocyanin label, an o-phthaldehyde label, a green fluorescent protein (GFP) label, and a fluoescamine label.

Examples of suitable toxin labels include diphtheria toxin, ricin, and cholera toxin.

5 Examples of chemiluminescent labels include a luminal label, an isoluminal label, an aromatic acridinium ester label, an imidazole label, an acridinium salt label, an oxalate ester label, a luciferin label, a luciferase label, and an aequorin label.

10 Examples of nuclear magnetic resonance contrasting agents include heavy metal nuclei such as Gd, Mn, and iron.

15 Typical techniques for binding the above-described labels to the antibodies of the invention are provided by Kennedy *et al.*, *Clin. Chim. Acta* 70:1-31 (1976), and Schurs *et al.*, *Clin. Chim. Acta* 81:1-40 (1977). Coupling techniques mentioned in the latter are the glutaraldehyde method, the periodate method, the dimaleimide method, the m-maleimidobenzyl-N-hydroxy-succinimide ester method, all of which methods are incorporated by reference herein.

20 It will be appreciated by one of ordinary skill that the antibodies of the present invention may alternatively be coupled to a solid support, to facilitate, for example, chromatographic and other immunological procedures using such solid phase-immobilized antibodies. Included among such procedures are the use of the antibodies of the invention to isolate or purify polypeptides comprising one or more epitopes encoded by the nucleic acid molecules of the invention (which may be fusion polypeptides or other polypeptides of the invention described herein), or to isolate or purify polynucleotides comprising one or more recombination site sequences of the invention or portions thereof. Methods for isolation and purification of polypeptides (and, by analogy, polynucleotides) by affinity chromatography, for example using the antibodies of the invention coupled to a solid phase support, are well-known in the art and will be familiar to one of ordinary skill. The antibodies of the invention may also be used in other applications, for example to cross-link or couple two or more proteins, polypeptides, polynucleotides, or portions thereof into a structural and/or functional complex. In one such use, an antibody of the invention may have two

25

30

or more distinct epitope-binding regions that may bind, for example, a first polypeptide (which may be a polypeptide of the invention) at one epitope-binding region on the antibody and a second polypeptide (which may be a polypeptide of the invention) at a second epitope-binding region on the antibody, thereby bringing the first and second polypeptides into close proximity to each other such that the first and second polypeptides are able to interact structurally and/or functionally (as, for example, linking an enzyme and its substrate to carry out enzymatic catalysis, or linking an effector molecule and its receptor to carry out or induce a specific binding of the effector molecule to the receptor or a response to the effector molecule mediated by the receptor). Additional applications for the antibodies of the invention include, for example, the preparation of large-scale arrays of the antibodies, polypeptides, or nucleic acid molecules of the invention, or portions thereof, on a solid support, for example to facilitate high-throughput screening of protein or RNA expression by host cells containing nucleic acid molecules of the invention (known in the art as "chip array" protocols; *see, e.g.*, U.S. Patent Nos. 5,856,101, 5,837,832, 5,770,456, 5,744,305, 5,631,734, and 5,593,839, which are directed to production and use of chip arrays of polypeptides (including antibodies) and polynucleotides, and the disclosures of which are incorporated herein by reference in their entireties). By "solid support" is intended any solid support to which an antibody can be immobilized. Such solid supports include, but are not limited to nitrocellulose, diazocellulose, glass, polystyrene, polyvinylchloride, polycarbonate, polypropylene, polyethylene, dextran, Sepharose, agar, starch, nylon, beads and microtitre plates. Preferred are beads made of glass, latex or a magnetic material. Linkage of an antibody of the invention to a solid support can be accomplished by attaching one or both ends of the antibody to the support. Attachment may also be made at one or more internal sites in the antibody. Multiple attachments (both internal and at the ends of the antibody) may also be used according to the invention. Attachment can be via an amino acid linkage group such as a primary amino group, a carboxyl group, or a sulfhydryl (SH) group or by chemical linkage groups such as with cyanogen bromide (CNBr) linkage through a spacer. For non-covalent attachments, addition of an affinity tag sequence to the peptide can be used such as GST

(Smith, D.B., and Johnson, K.S., *Gene* 67:31 (1988)), polyhistidines (Hochuli, E., *et al.*, *J. Chromatog.* 411:77 (1987)), or biotin. Alternatively, attachment can be accomplished using a ligand which binds the Fc region of the antibodies of the invention, *e.g.*, protein A or protein G. Such affinity tags may be used for the reversible attachment of the antibodies to the support. Peptides may also be recognized via specific ligand-receptor interactions or using phage display methodologies that will be familiar to the skilled artisan, for their ability to bind polypeptides of the invention or fragments thereof.

Kits

In another aspect, the invention provides kits which may be used in producing the nucleic acid molecules, polypeptides, vectors, host cells, and antibodies, and in the recombinational cloning methods, of the invention. Kits according to this aspect of the invention may comprise one or more containers, which may contain one or more of the nucleic acid molecules, primers, polypeptides, vectors, host cells, or antibodies of the invention. In particular, a kit of the invention may comprise one or more components (or combinations thereof) selected from the group consisting of one or more recombination proteins (*e.g.*, Int) or auxiliary factors (*e.g.* IHF and/or Xis) or combinations thereof, one or more compositions comprising one or more recombination proteins or auxiliary factors or combinations thereof (for example, GATEWAY™ LR Clonase™ Enzyme Mix or GATEWAY™ BP Clonase™ Enzyme Mix) one or more Destination Vector molecules (including those described herein), one or more Entry Clone or Entry Vector molecules (including those described herein), one or more primer nucleic acid molecules (particularly those described herein), one or more host cells (*e.g.* competent cells, such as *E. coli* cells, yeast cells, animal cells (including mammalian cells, insect cells, nematode cells, avian cells, fish cells, etc.), plant cells, and most particularly *E. coli* DB3, DB3.1 (preferably *E. coli* LIBRARY EFFICIENCY® DB3.1™ Competent Cells; Life Technologies, Inc., Rockville, MD), DB4 and DB5; *see* U.S. Provisional Application No. 60/122,392, filed on March 2, 1999, and the corresponding U.S. Utility Application No. _____ of Hartley *et al.*, entitled "Cells Resistant to Toxic Genes and Uses Thereof," filed

on even day herewith, the disclosures of which are incorporated by reference herein in its entirety), and the like. In related aspects, the kits of the invention may comprise one or more nucleic acid molecules encoding one or more recombination sites or portions thereof, such as one or more nucleic acid molecules comprising
5 a nucleotide sequence encoding the one or more recombination sites (or portions thereof) of the invention, and particularly one or more of the nucleic acid molecules contained in the deposited clones described herein. Kits according to this aspect of the invention may also comprise one or more isolated nucleic acid molecules of the invention, one or more vectors of the invention, one or more
10 primer nucleic acid molecules of the invention, and/or one or more antibodies of the invention. The kits of the invention may further comprise one or more additional containers containing one or more additional components useful in combination with the nucleic acid molecules, polypeptides, vectors, host cells, or antibodies of the invention, such as one or more buffers, one or more detergents,
15 one or more polypeptides having nucleic acid polymerase activity, one or more polypeptides having reverse transcriptase activity, one or more transfection reagents, one or more nucleotides, and the like. Such kits may be used in any process advantageously using the nucleic acid molecules, primers, vectors, host cells, polypeptides, antibodies and other compositions of the invention, for
20 example in methods of synthesizing nucleic acid molecules (e.g., via amplification such as via PCR), in methods of cloning nucleic acid molecules (preferably via recombinational cloning as described herein), and the like.

Optimization of Recombinational Cloning System

25 The usefulness of a particular nucleic acid molecule, or vector comprising a nucleic acid molecule, of the invention in methods of recombinational cloning may be determined by any one of a number of assay methods. For example, Entry and Destination vectors of the present invention may be assessed for their ability to function (*i.e.*, to mediate the transfer of a nucleic acid molecule, DNA segment,
30 gene, cDNA molecule or library from a cloning vector to an Expression Vector) by carrying out a recombinational cloning reaction as described in more detail in the Examples below and as described in U.S. Application Nos. 08/663,002, filed

June 7, 1996 (now U.S. Patent No. 5,888,732), 09/005,476, filed January 12, 1998, 09/177,387, filed October 23, 1998, and 60/108,324, filed November 13, 1998, the disclosures of which are incorporated by reference herein in their entireties. Alternatively, the functionality of Entry and Destination Vectors prepared according to the invention may be assessed by examining the ability of these vectors to recombine and create cointegrate molecules, or to transfer a nucleic acid molecule of interest, using an assay such as that described in detail below in Example 19. Analogously, the formulation of compositions comprising one or more recombination proteins or combinations thereof, for example GATEWAY™ LR Clonase™ Enzyme Mix and GATEWAY™ BP Clonase™ Enzyme Mix, may be optimized using assays such as those described below in Example 18.

Uses

There are a number of applications for the compositions, methods and kits of the present invention. These uses include, but are not limited to, changing vectors, targeting gene products to intracellular locations, cleaving fusion tags from desired proteins, operably linking nucleic acid molecules of interest to regulatory genetic sequences (*e.g.*, promoters, enhancers, and the like), constructing genes for fusion proteins, changing copy number, changing replicons, cloning into phages, and cloning, *e.g.*, PCR products, genomic DNAs, and cDNAs. In addition, the nucleic acid molecules, vectors, and host cells of the invention may be used in the production of polypeptides encoded by the nucleic acid molecules, in the production of antibodies directed against such polypeptides, in recombinational cloning of desired nucleic acid sequences, and in other applications that may be enhanced or facilitated by the use of the nucleic acid molecules, vectors, and host cells of the invention.

In particular, the nucleic acid molecules, vectors, host cells, polypeptides, antibodies, and kits of the invention may be used in methods of transferring one or more desired nucleic acid molecules or DNA segments, for example one or more genes, cDNA molecules or cDNA libraries, into a cloning or Expression Vector for use in transforming additional host cells for use in cloning or

amplification of, or expression of the polypeptide encoded by, the desired nucleic acid molecule or DNA segment. Such recombinational cloning methods which may advantageously use the nucleic acid molecules, vectors, and host cells of the invention, are described in detail in the Examples below, and in commonly owned
5 U.S. Application Nos. 08/486,139, filed June 7, 1995, 08/663,002, filed June 7, 1996 (now U.S. Patent No. 5,888,732), 09/005,476, filed January 12, 1998, 09/177,387, filed October 23, 1998, and 60/108,324, filed November 13, 1998, the disclosures of all of which are incorporated by reference herein in their entireties.

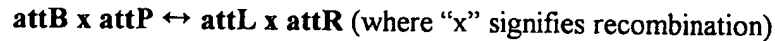
10 It will be understood by one of ordinary skill in the relevant arts that other suitable modifications and adaptations to the methods and applications described herein are readily apparent from the description of the invention contained herein in view of information known to the ordinarily skilled artisan, and may be made
15 without departing from the scope of the invention or any embodiment thereof. Having now described the present invention in detail, the same will be more clearly understood by reference to the following examples, which are included herewith for purposes of illustration only and are not intended to be limiting of the invention.

20 *Examples*

Example 1: Recombination Reactions of Bacteriophage λ

25 The *E. coli* bacteriophage λ can grow as a lytic phage, in which case the host cell is lysed, with the release of progeny virus. Alternatively, lambda can integrate into the genome of its host by a process called lysogenization (see Figure 60). In this lysogenic state, the phage genome can be transmitted to daughter cells for many generations, until conditions arise that trigger its excision from the genome.
30 At this point, the virus enters the lytic part of its life cycle. The control of the switch between the lytic and lysogenic pathways is one of the best understood processes in molecular biology (M. Ptashne, *A Genetic Switch*, Cell Press, 1992).

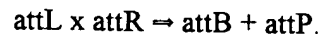
The integrative and excisive recombination reactions of λ , performed *in vitro*, are the basis of Recombinational Cloning System of the present invention. They can be represented schematically as follows:



10 The four att sites contain binding sites for the proteins that mediate the reactions. The wild type attP, attB, attL, and attR sites contain about 243, 25, 100, and 168 base pairs, respectively. The attB x attP reaction (hereinafter referred to as a "BP Reaction," or alternatively and equivalently as an "Entry Reaction" or a "Gateward Reaction") is mediated by the proteins Int and IHF. The attL x attR reaction (hereinafter referred to as an "LR Reaction," or alternatively and equivalently as a "Destination Reaction") is mediated by the proteins Int, IHF, and Xis. Int (integrase) and Xis (excisionase) are encoded by
15 the λ genome, while IHF (integration host factor) is an *E. coli* protein. For a general review of lambda recombination, see: A. Landy, *Ann. Rev. Biochem.* 58: 913-949 (1989).

20 ***Example 2: Recombination Reactions of the Recombinational Cloning System***

 The LR Reaction -- the exchange of a DNA segment from an Entry Clone to a Destination Vector -- is the *in vitro* version of the λ excision reaction:



30 There is a practical imperative for this configuration: after an LR Reaction in one configuration of the present method, an att site usually separates a functional motif (such as a promoter or a fusion tag) from a nucleic acid molecule of interest in an Expression Clone, and the 25 bp attB site is much smaller than the attP, attL, and attR sites.

 Note that the recombination reaction is conservative, i.e., there is no net synthesis or loss of base pairs. The DNA segments that flank the recombination

sites are merely switched. The wild type λ recombination sites are modified for purposes of the GATEWAY™ Cloning System, as follows:

To create certain preferred Destination Vectors, a part (43 bp) of attR was removed, to make the excisive reaction irreversible and more efficient (W. Bushman et al., *Science* 230: 906, 1985). The attR sites in preferred Destination Vectors of the invention are 125 bp in length. Mutations were made to the core regions of the att sites, for two reasons: (1) to eliminate stop codons, and (2) to ensure specificity of the recombination reactions (i.e., attR1 reacts only with attL1, attR2 reacts only with attL2, etc.).

Other mutations were introduced into the short (5 bp) regions flanking the 15 bp core regions of the attB sites to minimize secondary structure formation in single-stranded forms of attB plasmids, e.g., in phagemid ssDNA or in mRNA. Sequences of attB1 and attB2 to the left and right of a nucleic acid molecule of interest after it has been cloned into a Destination Vector are given in Figure 6.

Figure 61 illustrates how an Entry Clone and a Destination Vector recombine in the LR Reaction to form a co-integrate, which resolves through a second reaction into two daughter molecules. The two daughter molecules have the same general structure regardless of which pair of sites, attL1 and attR1 or attL2 and attR2, react first to form the co-integrate. The segments change partners by these reactions, regardless of whether the parental molecules are both circular, one is circular and one is linear, or both are linear. In this example, selection for ampicillin resistance carried on the Destination Vector, which also carries the death gene *ccdB*, provides the means for selecting only for the desired attB product plasmid.

Example 3: Protein Expression in the Recombinational Cloning System

Proteins are expressed *in vivo* as a result of two processes, transcription (DNA into RNA), and translation (RNA into protein). For a review of protein expression in prokaryotes and eukaryotes, see Example 13 below. Many vectors (pUC, BlueScript, pGem) use interruption of a transcribed *lacZ* gene for blue-white screening. These plasmids, and many Expression Vectors, use the *lac* promoter to control expression of cloned genes. Transcription from the *lac*

promoter is turned on by adding the inducer IPTG. However, a low level of RNA is made in the absence of inducer, i.e., the lac promoter is never completely off. The result of this "leakiness" is that genes whose expression is harmful to *E. coli* may prove difficult or impossible to clone in vectors that contain the lac promoter, or they may be cloned only as inactive mutants.

In contrast to other gene expression systems, nucleic acid molecules cloned into an Entry Vector may be designed *not* to be expressed. The presence of the strong transcriptional terminator *rrnB* (Orosz, et al., *Eur. J. Biochem.* 201: 653, 1991) just upstream of the attL1 site keeps transcription from the vector promoters (drug resistance and replication origin) from reaching the cloned gene. However, if a toxic gene is cloned into a Destination Vector, the host may be sick, just as in other expression systems. But the reliability of subcloning by *in vitro* recombination makes it easier to recognize that this has happened -- and easier to try another expression option in accordance with the methods of the invention, if necessary.

Example 4: Choosing the Right Entry Vector

There are two kinds of choices that must be made in choosing the best Entry Vector, dictated by (1) the particular DNA segment that is to be cloned, and (2) what is to be accomplished with the cloned DNA segment. These factors are critical in the choice of Entry Vector used, because when the desired nucleic acid molecule of interest is moved from the Entry Vector to a Destination Vector, all the base pairs between the nucleic acid molecule of interest and the Int cutting sites in attL1 and attL2 (such as in Figure 6) move into the Destination Vector as well. For genomic DNAs that are not expressed as a result of moving into a Destination Vector, these decisions are not as critical.

For example, if an Entry Vector with certain translation start signals is used, those sequences will be translated into amino acids if an amino-terminal fusion to the desired nucleic acid molecule of interest is made. Whether the desired nucleic acid molecule of interest is to be expressed as fusion protein, native protein, or both, dictates whether translational start sequences must be included between the attB sites of the clone (native protein) or, alternatively, supplied by the Destination

Vector (fusion protein). In particular, Entry Clones that include translational start sequences may prove less suitable for making fusion proteins, as internal initiation of translation at these sites can decrease the yield of N-terminal fusion protein. These two types of expression afforded by the compositions and methods of the invention are illustrated in Figure 62.

No Entry Vector is likely to be optimal for all applications. The nucleic acid molecule of interest may be cloned into any of several optimal Entry Vectors.

As an example, consider pENTR7 (Figure 16) and pENTR11 (Figure 20), which are useful in a variety of applications, including (but not limited to):

- Cloning cDNAs from most of the commercially available libraries. The sites to the left and right of the *ccdB* death gene have been chosen so that directional cloning is possible if the DNA to be cloned does not have two or more of these restriction sites.

- Cloning of genes directionally: *Sal*I, *Bam*HI, *Xmn*I (blunt), or *Kpn*I on the left of *ccdB*; *Not*I, *Xho*I, *Xba*I, or *Eco*RV (blunt), on the right.

- Cloning of genes or gene fragments with a blunt amino end at the *Xmn*I site. The *Xmn*I site has four of the six most favored bases for eukaryotic expression (*see* Example 13, below), so that if the first three bases of the DNA to be cloned are ATG, the open reading frame (ORF) will be expressed in eukaryotic cells (*e.g.*, mammalian cells, insect cells, yeast cells) when it is transcribed in the appropriate Destination Vector. In addition, in pENTR11, a Shine-Dalgarno sequence is situated 8 bp upstream, for initiating protein synthesis in a prokaryotic host cell (particularly a bacterial cell, such as *E. coli*) at an ATG.

- Cleaving off amino terminal fusions (*e.g.*, His₆, GST, or thioredoxin) using the highly specific TEV (Tobacco Etch Virus) protease (available from Life Technologies, Inc.). If the nucleic acid molecule of interest is cloned at the

blunt *Xmn*I site, TEV cleavage will leave two amino acids on the amino end of the expressed protein.

•Selecting against uncut or singly cut Entry Vector molecules during cloning with restriction enzymes and ligase. If the *ccdB* gene is not removed with a double digest, it will kill any recipient *E. coli* cell that does not contain a mutation that makes the cell resistant to *ccdB* (see U.S. Provisional Application No. 60/122,392, filed on March 2, 1999, the disclosure of which is incorporated by reference herein in its entirety).

•Allowing production of amino fusions with ORFs in all cloning sites. There are no stop codons (in the attL1 reading frame) upstream of the *ccdB* gene.

In addition, pENTR11 is also useful in the following applications:

•Cloning cDNAs that have an *Nco*I site at the initiating ATG into the *Nco*I site. Similar to the *Xmn*I site, this site has four of the six most favored bases for eukaryotic expression. Also, a Shine-Dalgarno sequence is situated 8 bp upstream, for initiating protein synthesis in a prokaryotic host cell (particularly a bacterial cell, such as *E. coli*) at an ATG.

•Producing carboxy fusion proteins with ORFs positioned in phase with the reading frame convention for carboxy-terminal fusions (see Figure 20A).

Table 1 lists some non-limiting examples of Entry Vectors and their characteristics, and Figures 10-20 show their cloning sites. All of the Entry Vectors listed in Table 1 are available commercially from Life Technologies, Inc., Rockville, Maryland. Other Entry Vectors not specifically listed here, which comprise alternative or additional features may be made by one of ordinary skill using routine methods of molecular and cellular biology, in view of the disclosure contained herein.

Table 1 Examples of Entry Vectors

Designation	Mnemonic Name	Class of Entry Vector	Distinctive Cloning Sites	Amino Fusions	Native Protein in E.coli	Native Protein in Eukaryotic Cells	Protein Synthesis Features
pENTR-1A, 2B, 3C	Minimal blunt RF A, B, C	Alternative Reading Frame Vectors	Reading frame A, B, or C; blunt cut closest to attL1	Good	Poor	Good	Minimal amino acids between tag and protein; no SD
pENTR4	Minimal Nco	Restr. Enz. Cleavage Vectors	Nco I site (common in euk. cDNAs) closest to attL1	Good	Poor	Good	Good Kozac; no SD
pENTR5	Minimal Nde	Restr. Enz. Cleavage Vectors	NdeI site closest to attL1	Good	Poor	Poor at Nde I, Good at Xmn I	No SD; poor Kozac at Nde, good at Xmn
pENTR6	Minimal Sph	Restr. Enz. Cleavage Vectors	Sph I site closest to attL1	Good	Poor	Poor at Sph I, Good at Xmn I	No SD; poor Kozac at Sph, good at Xmn
pENTR7	TEV Blunt	TEV Cleavage Site Present	Xmn I (blunt) is first cloning site after TEV site	Good	Poor	Good at Xmn I site	TEV protease leaves Gly-Thr on amino end of protein; no SD
pENTR8	TEV Nco	TEV Cleavage Site Present	Nco I is first cloning site after TEV site	Good	Poor	Good	TEV protease leaves Gly-Thr on amino end of protein; no SD

pENTR9	TEV Nde	TEV Cleavage Site Present	Nde I is first cloning site after TEV site	Good	Poor	Poor	TEV protease leaves Gly-Thr on amino end of protein; no SD, poor Kozac
pENTR10	Nde with SD	Good SD for E.coli Expression	Strong SD; Nde I site, no TEV	Poor	Good	Poor	Strong SD, internal starts in amino fusions. Poor Kz. No TEV
pENTR11	2 X SD+Kozac	Good SD for E.coli Expression	Xmn I (blunt) and Nco I sites each preceded by SD and Kozac	Good	Good	Good	Strong SD/Koz Internal starts in amino fusions. No TEV

Entry vectors pENTR1A (Figures 10A and 10B), pENTR2B (Figures 11A and 11B), and pENTR3C (Figures 12A and 12B) are almost identical, except that the restriction sites are in different reading frames. Entry vectors pENTR4 (Figures 13A and 13B), pENTR5 (Figures 14A and 14B), and pENTR6 (Figures 15A and 15B) are essentially identical to pENTR1A, except that the blunt *Dra*I site has been replaced with sites containing the ATG methionine codon: *Nco*I in pENTR4, *Nde*I in pENTR5, and *Sph*I in pENTR6. Nucleic acid molecules that contain one of these sites at the initiating ATG can be conveniently cloned in these Entry vectors. The *Nco*I site in pENTR4 is especially useful for expression of nucleic acid molecules in eukaryotic cells, since it contains many of the bases that give efficient translation (*see* Example 13, below). (Nucleic acid molecules of interest cloned into the *Nde*I site of pENTR5 are not expected to be highly expressed in eukaryotic cells, because the cytosine at position -3 from the initiating ATG is rare in eukaryotic genes.)

Entry vectors pENTR7 (Figures 16A and 16B), pENTR8 (Figures 17A and 17B), and pENTR9 (Figures 18A and 18B) contain the recognition site for the TEV protease between the attL1 site and the cloning sites. Cleavage sites for *Xmn*I (blunt), *Nco*I, and *Nde*I, respectively, are the most 5' sites in these Entry vectors. Amino fusions can be removed efficiently if nucleic acid molecules are cloned into these Entry vectors. TEV protease is highly active and highly specific.

Example 5: Controlling Reading Frame

One of the trickiest tasks in expression of cloned nucleic acid molecules is making sure the reading frame is correct. (Reading frame is important if fusions are being made between two ORFs, for example between a nucleic acid molecule of interest and a His6 or GST domain.) For purposes of the present invention, the following convention has been adopted: The reading frame of the DNA cloned into any Entry Vector must be in phase with that of the attB1 site shown in Figure 16A, pENTR7. Notice that the six As of the attL1 site are split into two lysine codons (aaa aaa). The Destination Vectors that make amino fusions were constructed such that they enter the attR1 site in this reading frame.

Destination Vectors for carboxy terminal fusions were also constructed, including those containing His₆ (pDEST23; Figure 43), GST (pDEST24; Figure 44), or thioredoxin (pDEST25; Figure 45) C-terminal fusion sequences.

Therefore, if a nucleic acid molecule of interest is cloned into an Entry Vector so that the aaa aaa reading frame within the attL1 site is in phase with the nucleic acid molecule's ORF, amino terminal fusions will automatically be correctly phased, for all the fusion tags. This is a significant improvement over the usual case, where each different vector can have different restriction sites and different reading frames.

See Example 15 for a practical example of how to choose the most appropriate combinations of Entry Vector and Destination Vector.

Materials

Unless otherwise indicated, the following materials were used in the remaining Examples included herein:

5X LR Reaction Buffer:

200-250 mM (preferably 250 mM) Tris-HCl, pH 7.5

250-350 mM (preferably 320 mM) NaCl

1.25-5 mM (preferably 4.75 mM) EDTA

12.5-35 mM (preferably 22-35 mM, and most preferably 35 mM)

Spermidine-HCl

1 mg/ml bovine serum albumin

GATEWAY™ LR Clonase™ Enzyme Mix:

per 4 µl of 1X LR Reaction Buffer:

150 ng carboxy-His6-tagged Int (see U.S. Appl. Nos. 60/108,324, filed November 13, 1998, and 09/438,358, filed November 12, 1999, both entirely incorporated by reference herein)

-106-

25 ng carboxy-His6-tagged Xis (see U.S. Appl. Nos. 60/108,324, filed
November 13, 1998, and 09/438,358, filed November 12,
1999, both entirely incorporated by reference herein)

30 ng IHF

50% glycerol

5X BP Reaction Buffer:

125 mM Tris-HCl, pH 7.5

110 mM NaCl

25 mM EDTA

25 mM Spermidine-HCl

5 mg/ml bovine serum albumin

GATEWAY™ BP Clonase™ Enzyme Mix:

per 4 µl of 1X BP Reaction Buffer:

200 ng carboxy-His6-tagged Int (see U.S. Appl. Nos. 60/108,324, filed
November 13, 1998, and 09/438,358, filed November 12,
1999, both entirely incorporated by reference herein)

80 ng IHF

50% glycerol

10X Clonase Stop Solution:

50 mM Tris-HCl, pH 8.0

1 mM EDTA

2 mg/ml Proteinase K

Example 6: LR ("Destination") Reaction

To create a new Expression Clone containing the nucleic acid molecule of
interest (and which may be introduced into a host cell, ultimately for production
of the polypeptide encoded by the nucleic acid molecule), an Entry Clone or
Vector containing the nucleic acid molecule of interest, prepared as described

herein, is reacted with a Destination Vector. In the present example, a β -Gal gene flanked by attL sites is transferred from an Entry Clone to a Destination Vector.

Materials needed:

- 5 X LR Reaction buffer
- Destination Vector (preferably linearized), 75-150 ng/ μ l
- Entry Clone containing nucleic acid molecule of interest, 100-300 ng in $\leq 8 \mu$ l TE buffer
- Positive control Entry Clone (pENTR- β -Gal) DNA (See note, below)
- Positive control Destination Vector, pDEST1 (pTrc), 75 ng/ μ l
- GATEWAY™ LR Clonase™ Enzyme Mix (stored at - 80° C)
- 10X Clonase Stop solution
- pUC19 DNA, 10 pg/ μ l
- Chemically competent *E. coli* cells (competence: $\geq 1 \times 10^7$ CFU/ μ g), 400 μ l.
- LB Plates containing ampicillin (100 μ g/ml) and methicillin (200 μ g/ml) \pm X-gal and IPTG (See below)

Notes:

Preparation of the Entry Clone DNA: Miniprep DNA that has been treated with RNase works well. A reasonably accurate quantitation ($\pm 50\%$) of the DNA to be cloned is advised, as the GATEWAY™ reaction appears to have an optimum of about 100-300 ng of Entry Clone per 20 μ l of reaction mix.

The positive control Entry Clone, pENTR- β -Gal, permits functional analysis of clones based on the numbers of expected blue vs. white colonies on LB plates containing IPTG + Bluo-gal (or X-gal), in addition to ampicillin (100 μ g/ml) and methicillin (200 μ g/ml). Because β -Galactosidase is a large protein, it often yields a less prominent band than many smaller proteins do on SDS protein gels.

In the Positive Control Entry Vector pENTR- β -Gal, the coding sequence of β -Gal has been cloned into pENTR11 (Figures 20A and 20B), with translational start signals permitting expression in *E. coli*, as well as in eukaryotic

cells. The positive control Destination Vector, for example pDEST1 (Figure 21), is preferably linearized.

To prepare X-gal + IPTG plates, either of the following protocols may be used:

5

A. With a glass rod, spread over the surface of an LB agar plate: 40 μ l of 20 mg/ml X-gal (or Bluo-gal) in DMF plus 4 μ l 200 mg/ml IPTG. Allow liquid to adsorb into agar for 3-4 hours at 37° C before plating cells.

10

B. To liquid LB agar at ~45° C, add: X-gal (or Bluo-Gal) (20 mg/ml in DMF) to make 50 μ g/ml and IPTG (200 mM in water) to make 0.5-1 mM, just prior to pouring plates. Store X-gal and Bluo-Gal in a light-shielded container.

15

Colony color may be enhanced by placing the plates at 5° C for a few hours after the overnight incubation at 37° C. Protocol B can give more consistent colony color than A, but A is more convenient when selection plates are needed on short notice.

20

Recombination in Clonase reactions continues for many hours. While incubations of 45-60 minutes are usually sufficient, reactions with large DNAs, or in which both parental DNAs are supercoiled, or which will be transformed into cells of low competence, can be improved with longer incubation times, such as 2-24 hours at 25° C.

Procedure:

25

1. Assemble reactions as follows (combine all components at room temperature, except GATEWAY™ LR Clonase™ Enzyme Mix ("Clonase LR"), before removing Clonase LR from frozen storage):

Component	Tube 1	Tube 2	Tube 3	Tube 4
	Neg.	Pos.	Neg.	Test
p-Gate-βGal, (Positive control Entry Clone) 75 ng/μl	4 μl	4 μl		
pDEST1 (Positive control Destination Vector), 75 ng/μl	4 μl	4 μl		
Your Entry Clone (100-300 ng)			1 - 8 μl	1 - 8 μl
Destination Vector for your nucleic acid molecule, 75 ng/μl			4 μl	4 μl
5 X LR Reaction Buffer	4 μl	4 μl	4 μl	4 μl
TE	8 μl	4 μl	To 20 μl	To 16 μl
GATEWAY™ LR Clonase™ Enzyme Mix (store at - 80° C, add last)	---	4 μl	---	4 μl
Total Volume	20 μl	20 μl	20 μl	20 μl

2. Remove the GATEWAY™ LR Clonase™ Enzyme Mix from the -80° C freezer, place immediately on ice. The Clonase takes only a few minutes to thaw.
3. Add 4 μl of GATEWAY™ LR Clonase™ Enzyme Mix to reactions #2 and #4;
4. Return GATEWAY™ LR Clonase™ Enzyme Mix to - 80° C freezer.
5. Incubate tubes at 25° for at least 60 minutes.
6. Add 2 μl Clonase Stop solution to all reactions. Incubate for 20 min at 37°C. (This step usually increases the total number of colonies obtained by 10-20 fold.)
7. Transform 2 μl into 100 μl competent *E. coli*. Select on plates containing ampicillin at 100 μg/ml.

Example 7: Transformation of *E. coli*

To introduce cloning or Expression Vectors prepared using the recombinational cloning system of the invention, any standard *E. coli* transformation protocol should be satisfactory. The following steps are recommended for best results:

1. Let the mixture of competent cells and Recombinational Cloning System reaction product stand on ice at least 15 minutes prior to the heat-shock step. This gives time for the recombination proteins to dissociate from the DNA, and improves the transformation efficiency.

2. Expect the reaction to be about 1%-5% efficient, i.e., 2 μ l of the reaction should contain at least 100 pg of the Expression Clone plasmid (taking into account the amounts of each parental plasmid in the reaction, and the subsequent dilution). If the E. coli cells have a competence of 10^7 CFU/ μ g, 100 pg of the desired clone plasmid will give about 1000 colonies, or more, if the entire transformation is spread on one ampicillin plate.

3. Always do a control pUC DNA transformation. If the number of colonies is not what you expect, the pUC DNA transformation gives you an indication of where the problem was.

Example 8: Preparation of attB-PCR Product

For preparation of attB-PCR products in the PCR cloning methods described in Example 9 below, PCR primers containing attB1 and attB2 sequences are used. The attB1 and attB2 primer sequences are as follows:

attB1: 5'-GGGGACAAGTTTGTACAAAAAAGCAGGCT-(template-specific sequence)-3'

attB2: 5'-GGGGACCACTTTGTACAAGAAAGCTGGGT-(template-specific sequence)-3'

The attB1 sequence should be added to the amino primer, and the attB2 sequence to the carboxy primer. The 4 guanines at the 5' ends of each of these primers enhance the efficiency of the minimal 25 bp attB sequences as substrates for use in the cloning methods of the invention.

Standard PCR conditions may be used to prepare the PCR product. The following suggested protocol employs PLATINUM *Taq* DNA Polymerase High

-111-

Fidelity®, available commercially from Life Technologies, Inc. (Rockville, MD). This enzyme mix eliminates the need for hot starts, has improved fidelity over Taq, and permits synthesis of a wide range of amplicon sizes, from 200 bp to 10 kb, or more, even on genomic templates.

Materials needed:

- PLATINUM Taq DNA Polymerase High Fidelity® (Life Technologies, Inc.)
- attB1- and attB2- containing primer pair (see above) specific for your template
- DNA template (linearized plasmid or genomic DNA)
- 10X High Fidelity PCR Buffer
- 10 mM dNTP mix
- PEG/MgCl₂ Mix (30% PEG 8000, 30 mM MgCl₂)

Procedure:

1.) Assemble the reaction as follows:

Component	Reaction with <u>Plasmid Target</u>	Reaction with <u>Genomic Target</u>
10X High Fidelity PCR Buffer	5 µl	5 µl
dNTP Mix 10 mM	1 µl	1 µl
MgSO ₄ , 50mM	2 µl	2 µl
attB1 Primer, 10 µM	2 µl	1 µl
attB2 Primer, 10 µM	2 µl	1 µl
Template DNA	1-5 ng*	≥ 100 ng
PLATINUM Taq High Fidelity	2 µl	1 µl
Water	to 50 µl	to 50 µl

* Use of higher amounts of plasmid template may permit fewer cycles (10-15) of PCR

2.) Add 2 drops mineral oil, as appropriate.

3.) Denature for 30 sec. at 94°C.

4.) Perform 25 cycles:

94°C for 15 sec-30 sec

55°C for 15 sec-30 sec

68°C for 1 min per kb of template.

5.) Following the PCR reaction, apply 1-2 µl of the reaction mixture to an agarose gel, together with size standards (*e.g.*, 1 Kb Plus Ladder, Life Technologies, Inc.) and quantitation standards (*e.g.*, Low Mass Ladder, Life Technologies, Inc.), to assess the yield and uniformity of the product.

Purification of the PCR product is recommended, to remove attB primer dimers which can clone efficiently into the Entry Vector. The following protocol is fast and will remove DNA <300 bp in size:

6.) Dilute the 50 µl PCR reaction to 200 µl with TE.

7.) Add 100 µl PEG/MgCl₂ Solution. Mix and centrifuge immediately at 13,000 RPM for 10 min at room temperature. Remove the supernatant (pellet is clear and hard to see).

8.) Dissolve the pellet in 50 µl TE and check recovery on a gel.

If the starting PCR template is a plasmid that contains the gene for Kan^r, it is advisable to treat the completed PCR reaction with the restriction enzyme *DpnI*, to degrade the plasmid since unreacted residual starting plasmid is a potential source of false-positive colonies from the transformation of the GATEWAY™ Cloning System reaction. Adding ~5 units of *DpnI* to the completed PCR reaction and incubating for 15 min at 37°C will eliminate this potential problem. Heat inactivate the *DpnI* at 65°C for 15 min, prior to using the PCR product in the GATEWAY™ Cloning System reaction.

Example 9: Cloning attB-PCR products into Entry Vectors via the BP ("Gateway") Reaction

The addition of 5'-terminal attB sequences to PCR primers allows synthesis of a PCR product that is an efficient substrate for recombination with a Donor (attP) Plasmid in the presence of GATEWAY™ BP Clonase™ Enzyme Mix. This reaction produces an Entry Clone of the PCR product (See Figure 8).

The conditions of the Gateway Cloning reaction with an attB PCR substrate are similar to those of the BP Reaction (see Example 10 below), except that the attB-PCR product (see Example 8) substitutes for the Expression Clone, and the attB-PCR positive control (attB-tet^r) substitutes for the Expression Clone Positive Control (GFP).

Materials needed:

- 5 X BP Reaction Buffer
- Desired attB-PCR product DNA, 50-100 ng in $\leq 8 \mu\text{l}$ TE.
- Donor (attP) Plasmid (Figures 49-54), 75 ng/ μl , supercoiled DNA
- attB-tet^r PCR product positive control, 25 ng/ μl
- GATEWAY™ BP Clonase™ Enzyme Mix (stored at - 80° C)
- 10x Clonase Stop Solution
- pUC19 DNA, 10 pg/ μl .
- Chemically competent E.coli cells (competence: $\geq 1 \times 10^7$ CFU/ μg), 400 μl

Notes:

- Preparation of attB-PCR DNA: see Example 8.
- The Positive Control attB-tet^r PCR product contains a functional copy of the tet^r gene of pBR322, with its own promoter. By plating the transformation of the control BP Reaction on kanamycin (50 $\mu\text{g/ml}$) plates (if kan^r Donor Plasmids are used; see Figures 49-52) or an alternative selection agent (*e.g.*, gentamycin, if gen^r Donor Plasmids are used; see Figure 54), and then picking about 50 of these colonies onto plates with tetracycline (20 $\mu\text{g/ml}$), the

percentage of Entry Clones containing functional tet^r among the colonies from the positive control reaction can be determined (% Expression Clones = (number of tet^r + kan^r (or gen^r) colonies/kan^r (or gen^r) colonies).

Procedure:

1. Assemble reactions as follows. Combine all components except GATEWAY™ BP Clonase™ Enzyme Mix, before removing GATEWAY™ BP Clonase™ Enzyme Mix from frozen storage.

	Neg.	Pos.	Test
Component	Tube 1	Tube 2	Tube 3
attB-PCR product, 50-100 ng			1 - 8 µl
Donor (attP) Plasmid 75 ng/µl	2 µl	2 µl	2 µl
attB-PCR tet ^r control DNA (75 ng/µl)		4 µl	
5 X BP Reaction Buffer	4 µl	4 µl	4 µl
TE	10 µl	6 µl	To 16 µl
GATEWAY™ BP Clonase™ Enzyme Mix (store at -80° C, add last)	4 µl	4 µl	4 µl
Total Volume	20 µl	20 µl	20 µl

2. Remove the GATEWAY™ BP Clonase™ Enzyme Mix from the -80° C freezer, place immediately on ice. The Clonase takes only a few minutes to thaw.
3. Add 4 µl of GATEWAY™ BP Clonase™ Enzyme Mix to the subcloning reaction, mix.
4. Return GATEWAY™ BP Clonase™ Enzyme Mix to - 80° C freezer.
5. Incubate tubes at 25° for at least 60 minutes.

6. Add 2 μ l Proteinase K (2 μ g/ μ l) to all reactions. Incubate for 20 min at 37°C.
7. Transform 2 μ l into 100 μ l competent *E. coli*, as per 3.2, above. Select on LB plates containing kanamycin, 50 μ g/ml.

Results:

In initial experiments, primers for amplifying tetR and ampR from pBR322 were constructed containing only the tetR- or ampR-specific targeting sequences, the targeting sequences plus attB1 (for forward primers) or attB2 (for reverse primers) sequences shown in Figure 9, or the attB1 or attB2 sequences with a 5' tail of four guanines. The construction of these primers is depicted in Figure 65. After PCR amplification of tetR and ampR from pBR322 using these primers and cloning the PCR products into host cells using the recombinational cloning system of the invention, the results shown in Figure 66 were obtained. These results demonstrated that primers containing attB sequences provided for a somewhat higher number of colonies on the tetracycline and ampicillin plates. However, inclusion of the 5' extensions of four or five guanines on the primers in addition to the attB sequences provided significantly better cloning results, as shown in Figures 66 and 67. These results indicate that the optimal primers for cloning of PCR products using recombinational cloning will contain the recombination site sequences with a 5' extension of four or five guanine bases.

To determine the optimal stoichiometry between attB-containing PCR products and attP-containing Donor plasmid, experiments were conducted where the amount of PCR product and Donor plasmid were varied during the BP Reaction. Reaction mixtures were then transformed into host cells and plated on tetracycline plates as above. Results are shown in Figure 68. These results indicate that, for optimal recombinational cloning results with a PCR product in the size range of the tet gene, the amounts of attP-containing Donor plasmids are between about 100-500 ng (most preferably about 200-300 ng), while the optimal concentrations of attB-containing PCR products is about 25-100 ng (most preferably about 100 ng), per 20 μ l reaction.

Experiments were then conducted to examine the effect of PCR product size on efficiency of cloning via the recombinational cloning approach of the invention.

PCR products containing attB1 and attB2 sites, at sizes 256 bp, 1 kb, 1.4 kb, 3.4 kb, 4.6 kb, 6.9 kb and 10.1 kb were prepared and cloned into Entry vectors as described above, and host cells were transformed with the Entry vectors containing the cloned PCR products. For each PCR product, cloning efficiency was calculated relative to cloning of pUC19 positive control plasmids as follows:

$$\text{Cloning Efficiency} = \frac{\text{CFU/ng attB PCR product}}{\text{CFU/ng pUC19 control}} \times \frac{\text{Size (kb) PCR product}}{\text{Size (kb) pUC19 control}}$$

The results of these experiments are depicted in Figures 69A-69C (for 256 bp PCR fragments), 70A-70C (for 1 kb PCR fragments), 71A-71C (for 1.4 kb PCR fragments), 72A-72C (for 3.4 kb PCR fragments), 73A-73C (for 4.6 kb PCR fragments), 74 (for 6.9 kb PCR fragments), and 75-76 (for 10.1 kb PCR fragments). The results shown in these figures are summarized in Figure 77, for different weights and moles of input PCR DNA.

Together, these results demonstrate that attB-containing PCR products ranging in size from about 0.25 kb to about 5 kb clone relatively efficiently in the recombinational cloning system of the invention. While PCR products larger than about 5 kb clone less efficiently (apparently due to slow resolution of cointegrates), longer incubation times during the recombination reaction appears to improve the efficiency of cloning of these larger PCR fragments. Alternatively, it may also be possible to improve efficiency of cloning of large (> about 5 kb) PCR fragments by using lower levels of input attP Donor plasmid and perhaps attB-containing PCR product, and/or by adjusting reaction conditions (e.g., buffer conditions) to favor more rapid resolution of the cointegrates.

Example 10: The BP Reaction

One purpose of the Gateway ("Entry") reaction is to convert an Expression Clone into an Entry Clone. This is useful when you have isolated an individual Expression Clone from an Expression Clone cDNA library, and you wish to transfer the nucleic acid molecule of interest into another Expression Vector, or

to move a population of molecules from an attB or attL library. Alternatively, you may have mutated an Expression Clone and now wish to transfer the mutated nucleic acid molecule of interest into one or more new Expression Vectors. In both cases, it is necessary first to convert the nucleic acid molecule of interest to an Entry Clone.

Materials needed:

- 5 X BP Reaction Buffer
- Expression Clone DNA, 100-300 ng in $\leq 8 \mu\text{l}$ TE.
- Donor (attP) Vector, 75 ng/ μl , supercoiled DNA
- Positive control attB-tet-PCR DNA, 25 ng/ μl
- GATEWAY™ BP Clonase™ Enzyme Mix (stored at -80°C)
- Clonase Stop Solution (Proteinase K, 2 $\mu\text{g}/\mu\text{l}$).

Notes:

Preparation of the Expression Clone DNA: Miniprep DNA treated with RNase works well.

1. As with the LR Reaction (see Example 14), the BP Reaction is strongly influenced by the topology of the reacting DNAs. In general, the reaction is most efficient when one of the DNAs is linear and the other is supercoiled, compared to reactions where the DNAs are both linear or both supercoiled. Further, linearizing the attB Expression Clone (anywhere within the vector) will usually give more colonies than linearizing the Donor (attP) Plasmid. If finding a suitable cleavage site within your Expression Clone vector proves difficult, you may linearize the Donor (attP) Plasmid between the attP1 and attP2 sites (for example, at the *NcoI* site), avoiding the *ccdB* gene. Maps of Donor (attP) Plasmids are given in Figures 49-54.

Procedure:

1. Assemble reactions as follows. Combine all components at room temperature, except GATEWAY™ BP Clonase™ Enzyme Mix, before removing GATEWAY™ BP Clonase™ Enzyme Mix from freezer.

Component	Neg.	Pos.	Test
	Tube 1	Tube 2	Tube 3
Positive Control, attB-tet-PCR DNA, 25 ng/μl	4 μl	4 μl	
Desired attB Expression Clone DNA (100ng) linearized			1 - 8 μl
Donor (attP) Plasmid, 75 ng/μl	2 μl	2 μl	2 μl
5 X BP Reaction Buffer	4 μl	4 μl	4 μl
TE	10 μl	6 μl	To 16 μl
GATEWAY™ BP Clonase™ Enzyme Mix (store at - 80° C, add last)	---	4 μl	4 μl
Total Volume	20 μl	20 μl	20 μl

2. Remove the GATEWAY™ BP Clonase™ Enzyme Mix from the -80° C freezer, place immediately on ice. The mixture takes only a few minutes to thaw.
3. Add 4 μl of GATEWAY™ BP Clonase™ Enzyme Mix to the subcloning reaction, mix.
4. Return GATEWAY™ BP Clonase™ Enzyme Mix to - 80° C freezer.
5. Incubate tubes at 25° for at least 60 minutes. If both the attB and attP DNAs are supercoiled, incubation for 2-24 hours at 25° C is recommended.
6. Add 2 μl Clonase Stop Solution. Incubate for 10 min at 37° C.
7. Transform 2 μl into 100 μl competent E. coli, as above. Select on LB plates containing 50 μg/ml kanamycin.

Example 11: Cloning PCR Products into Entry Vectors using Standard Cloning Methods

Preparation of Entry Vectors for Cloning of PCR Products

All of the Entry Vectors of the invention contain the death gene *ccdB* as a stuffer between the "left" and "right" restriction sites. The advantage of this arrangement is that there is virtually no background from vector that has not been cut with both restriction enzymes, because the presence of the *ccdB* gene will kill

all standard E. coli strains. Thus it is necessary to cut each Entry Vector twice, to remove the ccdB fragment.

We strongly recommend that, after digestion of the Entry Vector with the second restriction enzyme, you treat the reaction with phosphatase (calf intestine alkaline phosphatase, CIAP or thermosensitive alkaline phosphatase, TSAP). The phosphatase can be added directly to the reaction mixture, incubated for an additional time, and inactivated. This step dephosphorylates both the vector and ccdB fragments, so that during subsequent ligation there is less competition between the ccdB fragment and the DNA of interest for the termini of the Entry Vector.

Blunt Cloning of PCR products

Generally PCR products do not have 5' phosphates (because the primers are usually 5' OH), and they are not necessarily blunt. (On this latter point, see Brownstein, et al., *BioTechniques* 20: 1006, 1996 for a discussion of how the sequence of the primers affects the addition of single 3' bases.) The following protocol repairs these two defects.

In a 0.5 ml tube, ethanol precipitate about 40 ng of PCR product (as judged from an agarose gel).

1. Dissolve the precipitated DNA in 10 µl comprising 1 µl 10 mM rATP, 1 µl mixed 2 mM dNTPs (i.e., 2 mM each dATP, dCTP, dTTP, and dGTP), 2 µl 5x T4 polynucleotide kinase buffer (350 mM Tris HCl (pH7.6), 50 mM MgCl₂, 500mM KCl, 5 mM 2-mercaptoethanol) 10 units T4 polynucleotide kinase, 1 µl T4 DNA polymerase, and water to 10 µl.
2. Incubate the tube at 37° for 10 minutes, then at 65° for 15 minutes, cool, centrifuge briefly to bring any condensate to the tip of the tube.
3. Add 5 µl of the PEG/MgCl₂ solution, mix and centrifuge at room temperature for 10 minutes. Discard supernatant.
4. Dissolve the invisible precipitate in 10 µl containing 2 µl 5x T4 DNA ligase buffer (Life Technologies, Inc.), 0.5 units T4 DNA ligase, and about 50 ng of blunt, phosphatase-treated Entry Vector.

5. Incubate at 25° for 1 hour, then 65° for 10 minutes. Add 90 µl TE, transform 10 µl into 50 - 100 µl competent *E. coli* cells.
6. Plate on kanamycin.

5 **Note:** In the above protocol, steps b-c simultaneously polish the ends of the PCR product (through the exonuclease and polymerase activities of T4 DNA polymerase) and phosphorylate the 5' ends (using T4 polynucleotide kinase). It is necessary to inactivate the kinase, so that the blunt, dephosphorylated vector in step e cannot self ligate. Step d (the PEG precipitation) removes all small
10 molecules (primers, nucleotides), and has also been found to improve the yield of cloned PCR product by 50 fold.

Cloning PCR Products after Digestion with Restriction Enzymes

Efficient cloning of PCR products that have been digested with restriction
15 enzymes includes three steps: inactivation of *Taq* DNA polymerase, efficient restriction enzyme cutting, and removal of small DNA fragments.

Inactivation of Taq DNA Polymerase: Carryover of *Taq* DNA polymerase and dNTPs into a RE digestion significantly reduces the success in cloning a PCR product (D. Fox et al., *FOCUS* 20(1):15, 1998), because *Taq* DNA polymerase
20 can fill in sticky ends and add bases to blunt ends. Either TAQQUENCH™ (obtainable from Life Technologies, Inc.; Rockville, Maryland) or extraction with phenol can be used to inactivate the *Taq*.

Efficient Restriction Enzyme Cutting: Extra bases on the 5' end of each PCR primer help the RE cut near ends of PCR products. With the availability of
25 cheap primers, adding 6 to 9 bases on the 5' sides of the restriction sites is a good investment to ensure that most of the ends are digested. Incubation of the DNA with a 5-fold excess of restriction enzyme for an hour or more helps ensure success.

Removal of Small Molecules before Ligation: Primers, nucleotides,
30 primer dimers, and small fragments produced by the restriction enzyme digestion,

can all inhibit or compete with the desired ligation of the PCR product to the cloning vector. This protocol uses PEG precipitation to remove small molecules.

Protocol for cutting the ends of PCR products with restriction enzyme(s):

1. Inactivation of Taq DNA polymerase in the PCR product:

Option A: Extraction with Phenol

A1. Dilute the PCR reaction to 200 μ l with TE. Add an equal volume of phenol:chloroform:isoamyl alcohol, vortex vigorously for 20 seconds, and centrifuge for 1 minute at room temperature. Discard the lower phase.

A2. Extract the phenol from the DNA and concentrate as follows. Add an equal volume of 2-butanol (colored red with "Oil Red O" from Aldrich, if desired), vortex briefly, centrifuge briefly at room temperature. Discard the upper butanol phase. Repeat the extraction with 2-butanol. This time the volume of the lower aqueous phase should decrease significantly. Discard the upper 2-butanol phase.

A3. Ethanol precipitate the DNA from the aqueous phase of the above extractions. Dissolve in a 200 μ l of a suitable restriction enzyme (RE) buffer.

Option B: Inactivation with TaqQuench

B1. Ethanol precipitate an appropriate amount of PCR product (100 ng to 1 μ g), dissolve in 200 μ l of a suitable RE buffer.

B2. Add 2 μ l TaqQuench.

2. Add 10 to 50 units of restriction enzyme and incubate for at least 1 hour. Ethanol precipitate if necessary to change buffers for digestion at the other end of the PCR product.

3. Add ½ volume of the PEG/MgCl₂ mix to the RE digestion. Mix well and immediately centrifuge at room temperature for 10 minutes. Discard the supernatant (pellet is usually invisible), centrifuge again for a few seconds, discard any remaining supernatant.

4. Dissolve the DNA in a suitable volume of TE (depending on the amount of PCR product in the original amplification reaction) and apply an aliquot to an agarose gel to confirm recovery. Apply to the same gel 20-100 ng of the appropriate Entry Vector that will be used for the cloning.

Example 12: Determining The Expected Size of the GATEWAY™ Cloning Reaction Products

If you have access to a software program that will electronically cut and splice sequences, you can create electronic clones to aid you in predicting the sizes and restriction patterns of GATEWAY™ Cloning System recombination products.

The cleavage and ligation steps performed by the enzyme Int in the GATEWAY™ Cloning System recombination reactions mimic a restriction enzyme cleavage that creates a 7-bp 5'-end overhang followed by a ligation step that reseals the ends of the daughter molecules. The recombination proteins present in the Clonase cocktails (see Example 19 below) recognize the 15 bp core sequence present within all four types of att sites (in addition to other flanking sequences characteristic of each of the different types of att sites).

By treating these sites in your software program as if they were restriction sites, you can cut and splice your Entry Clones with various Destination Vectors and obtain accurate maps and sequences of the expected results from your GATEWAY™ Cloning System reactions.

Example 13: Protein Expression

Brief Review of Protein Expression

Transcription: The most commonly used promoters in *E. coli* Expression Vectors are variants of the lac promoter, and these can be turned on by adding

IPTG to the growth medium. It is usually good to keep promoters off until expression is desired, so that the host cells are not made sick by the overabundance of some heterologous protein. This is reasonably easy in the case of the lac promoters used in *E. coli*. One needs to supply the *lac I* gene (or its more productive relative, the *lac I^q* gene) to make *lac* repressor protein, which binds near the promoter and keeps transcription levels low. Some Destination Vectors for *E. coli* expression carry their own *lacI^q* gene for this purpose. (However, lac promoters are always a little "on," even in the absence of IPTG.)

Controlling transcription in eukaryotic cells is not nearly so straightforward or efficient. The tetracycline system of Bujard and colleagues is the most successful approach, and one of the Destination Vectors (pDEST11; Figure 31) has been constructed to supply this function.

Translation: Ribosomes convert the information present in mRNA into protein. Ribosomes scan RNA molecules looking for methionine (AUG) codons, which begin nearly all nascent proteins. Ribosomes must, however, be able to distinguish between AUG codons that code for methionine in the middle of proteins from those at the start. Most often ribosomes choose AUGs that are 1) first in the RNA (toward the 5' end), and 2) have the proper sequence context. In *E. coli* the favored context (first recognized by Shine and Dalgarno, *Eur. J. Biochem.* 57: 221 (1975)) is a run of purines (As and Gs) from five to 12 bases upstream of the initiating AUG, especially AGGAGG or some variant.

In eukaryotes, a survey of translated mRNAs by Kozak (*J. Biol. Chem.* 266: 19867 (1991)) has revealed a preferred sequence context, gcc Acc ATGG, around the initiating methionine, with the A at -3 being most important, and a purine at +4 (where the A of the ATG is +1), preferably a G, being next most influential. Having an A at -3 is enough to make most ribosomes choose the first AUG of an mRNA, in plants, insects, yeast, and mammals. (For a review of initiation of protein synthesis in eukaryotic cells, see: Pain, V.M. *Eur. J. Biochem.* 236:747-771, 1996.)

Consequences of Translation Signals for GATEWAY™ Cloning System: First, translation signals (Shine-Dalgarno in *E. coli*, Kozak in eukaryotes) have to be close to the initiating ATG. The attB site is 25 base pairs long. Thus if

translation signals are desired near the natural ATG of the nucleic acid molecule of interest, they must be present in the Entry Clone of that nucleic acid molecule of interest. Also, when a nucleic acid molecule of interest is moved from an Entry Clone to a Destination vector, any translation signals will move along. The result is that the presence or absence of Shine-Dalgarno and/or Kozak sequences in the Entry Clone must be considered, with the eventual Destination Vectors to be used in mind.

Second, although ribosomes choose the 5' ATG most often, internal ATGs are also used to begin protein synthesis. The better the translation context around this internal ATG, the more internal translation initiation will be seen. This is important in the GATEWAY™ Cloning System, because you can make an Entry Clone of your nucleic acid molecule of interest, and arrange to have Shine-Dalgarno and/or Kozak sequences near the ATG. When this cassette is recombined into a Destination Vector that transcribes your nucleic acid molecule of interest, you get native protein. If you want, you can make a fusion protein in a different Destination Vector, since the Shine-Dalgarno and/or Kozak sequences do not contain any stop signals in the same reading frame. However, the presence of these internal translation signals may result in a significant amount of native protein being made, contaminating, and lowering the yield of, your fusion protein. This is especially likely with short fusion tags, like His6.

A good compromise can be recommended. If an Entry Vector like pENTR7 (Figure 16) or pENTR8 (Figure 17) is chosen, the Kozak bases are present for native eukaryotic expression. The context for *E. coli* translation is poor, so the yield of an amino-terminal fusion should be good, and the fusion protein can be digested with the TEV protease to make near-native protein following purification.

Recommended Conditions for Synthesis of Proteins in E. coli: When making proteins in *E. coli* it is advisable, at least initially, to incubate your cultures at 30°C, instead of at 37°C. Our experience indicates that proteins are less likely to form aggregates at 30°C. In addition, the yields of proteins from cells grown at 30°C frequently are improved.

The yields of proteins that are difficult to express may also be improved by inducing the cultures in mid-log phase of growth, using cultures begun in the morning from overnight growths, as opposed to harvesting directly from an overnight culture. In the latter case, the cells are preferably in late log or stationary growth, which can favor the formation of insoluble aggregates.

Example 14: Constructing Destination Vectors from Existing Vectors

Destination Vectors function because they have two recombination sites, attR1 and attR2, flanking a chloramphenicol resistance (CmR) gene and a death gene, ccdB. The GATEWAY™ Cloning System recombination reactions exchange the entire Cassette (except for a few bases comprising part of the attB sites) for the DNA segment of interest from the Entry Vector. Because attR1, CmR, ccdB gene, and attR2 are contiguous, they can be moved on a single DNA segment. If this Cassette is cloned into a plasmid, the plasmid becomes a Destination Vector. Figure 63 shows a schematic of the GATEWAY™ Cloning System Cassette; attR cassettes in all three reading frames contained in vectors pEYC15101, pEYC15102 and pEYC15103 are shown in Figures 64A, 64B, and 64C, respectively.

The protocol for constructing a Destination Vector is presented below. Keep in mind the following points:

- Destination Vectors must be constructed and propagated in one of the DB strains of *E. coli* (e.g., DB3.1, and particularly *E. coli* LIBRARY EFFICIENCY® DB3.1™ Competent Cells) available from Life Technologies, Inc. (and described in detail in U.S. Provisional Application No. 60/122,392, filed on March 2, 1999, which is incorporated herein by reference), because the ccdB death gene will kill any *E. coli* strain that has not been mutated such that it will survive the presence of the ccdB gene.
- If your Destination Vector will be used to make a fusion protein, a GATEWAY™ Cloning System cassette with the correct reading frame must be used. The nucleotide sequences of the ends of the cassettes are shown in Figure 78. The reading frame of the fusion protein domain must

be in frame with the core region of the attR1 site (for an amino terminal fusion) so that the six As are translated into two lysine codons. For a C-terminal fusion protein, translation through the core region of the attR2 site should be in frame with -TAC-AAA-, to yield -Tyr-Lys-.

- Note that each reading frame Cassette has a different unique restriction site between the chloramphenicol resistance and ccdB genes (*Mlu*I for reading frame A, *Bgl*II for reading frame B, and *Xba*I for reading frame C; see Figure 63).
- Most standard vectors can be converted to Destination Vectors, by inserting the Entry Cassette into the MCS of that vector.

Protocol for Making a Destination Vector

1. If the vector will make an amino fusion protein, it is necessary to keep the “aaa” triplets in attR1 in phase with the triplets of the fusion protein. Determine which Entry cassette to use as follows:

a.) Write out the nucleotide sequence of the existing vector near the restriction site into which the Entry cassette will be cloned. These must be written in triplets corresponding to the amino acid sequence of the fusion domain.

b.) Draw a vertical line through the sequence that corresponds to the restriction site end, after it has been cut and made blunt, i.e., after filling in a protruding 5' end or polishing a protruding 3' end.

c.) Choose the appropriate reading frame cassette:

- If the coding sequence of the blunt end ends after a complete codon triplet, use the reading frame A cassette. See Figures 78, 79 and 80.

- If the coding sequence of the blunt end ends in a single base, use the reading frame B cassette. See Figures 78, 79 and 81.

- If the coding sequence of the blunt end ends in two bases, use the reading frame C cassette. See Figures 78, 79, 82A-B, and 83A-C.

2. Cut one to five micrograms of the existing plasmid at the position where you wish your nucleic acid molecule of interest (flanked by att sites) to be after the recombination reactions. **Note:** it is better to remove as many of the MCS restriction sites as possible at this step. This makes it more likely that restriction enzyme sites within the GATEWAY™ Cloning System Cassette will be unique in the new plasmid, which is important for linearizing the Destination Vector (Example 14, below).

3. Remove the 5' phosphates with alkaline phosphatase. While this is not mandatory, it increases the probability of success.

4. Make the end(s) blunt with fill-in or polishing reactions. For example, to 1 µg of restriction enzyme-cut, ethanol-precipitated vector DNA, add:

- 20 µl 5x T4 DNA Polymerase Buffer (165 mM Tris-acetate (pH 7.9), 330 mM Na acetate, 50 mM Mg acetate, 500 µg/ml BSA, 2.5 mM DTT)
- 5 µl 10mM dNTP mix
- 1 Unit of T4 DNA Polymerase
- Water to a final volume of 100 µl
- Incubate for 15 min at 37°C.

5. Remove dNTPs and small DNA fragments: Ethanol precipitate (add three volumes of room temperature ethanol containing 0.1 M sodium acetate, mix well, immediately centrifuge at room temperature 5 - 10 minutes), dissolve wet precipitate in 200 µl TE, add 100 µl 30% PEG 8000, 30 mM MgCl₂, mix well,

immediately centrifuge for 10 minutes at room temperature, discard supernatant, centrifuge again a few seconds, discard any residual liquid.

5 6. Dissolve the DNA to a final concentration of 10 - 50 ng per microliter. Apply 20 - 100 ng to a gel next to supercoiled plasmid and linear size standards to confirm cutting and recovery. The cutting does not have to be 100% complete, since you will be selecting for the chloramphenicol marker on the Entry cassette.

10 7. In a 10 μ l ligation reaction combine 10 - 50 ng vector, 10 - 20 ng of Entry Cassette (Figure 79), and 0.5 units T4 DNA ligase in ligase buffer. After one hour (or overnight, whichever is most convenient), transform 1 μ l into one of the DB strains of competent *E. coli* cells with a *gyrA462* mutation (See U.S. Provisional Application No. 60/122,392, filed on March 2, 1999, which is incorporated herein by reference), preferably DB3.1, and most preferably *E. coli* LIBRARY
15 EFFICIENCY@DB3.1™ Competent Cells. The *ccdB* gene on the Entry Cassette will kill other strains of *E. coli* that have not been mutated so as to survive the presence of the *ccdB* gene.

20 8. After expression in SOC medium, plate 10 μ l and 100 μ l on chloramphenicol-containing (30 μ g / ml) plates, incubate at 37° C.

25 9. Pick colonies, make miniprep DNA. Treat the miniprep with RNase A and store in TE. Cut with the appropriate restriction enzyme to determine the orientation of the Cassette. Choose clones with the attR1 site next to the amino end of the protein expression function of the plasmid.

Notes on Using Destination Vectors

- We have found that about ten-fold more colonies result from a GATEWAY™ Cloning System reaction if the Destination Vector is linear or relaxed. If the
30 competent cells you use are highly competent ($>10^8$ per microgram), linearizing the Destination Vector is less essential.

- The site or sites used for the linearization must be within the Entry Cassette. Sites that cut once or twice within each cassette are shown in Figures 80-82.
- Minipreps of Destination Vectors will work fine, so long as they have been treated with RNase. Since most DB strains are *endA*- (See U.S. Provisional Application No. 60/122,392, filed on March 2, 1999, which is incorporated herein by reference), minipreps can be digested with restriction enzymes without a prior phenol extraction.
- Reading the OD₂₆₀ of miniprep DNA is inaccurate unless the RNA and ribonucleotides have been removed, for example, by a PEG precipitation.

Example 15: Some Options in Choosing Appropriate Entry Vectors and Destination Vectors: An Example

In some applications, it may be desirable to express a nucleic acid molecule of interest in two forms: as an amino-terminal fusion in *E. coli*, and as a native protein in eukaryotic cells. This may be accomplished in any of several ways:

Option 1: Your choices depend on your nucleic acid molecule of interest and the fragment that contains it, as well as the available Entry Vectors. For eukaryotic translation, you need consensus bases according to Kozak (*J. Biol. Chem.* 266:19867, 1991) near the initiating methionine (ATG) codon. All of the Entry Vectors offer this motif upstream of the *XmnI* site (blunt cutter). One option is to amplify your nucleic acid molecule of interest, with its ATG, by PCR, making the amino end blunt and the carboxy end containing the natural stop codon followed by one of the "right side" restriction sites (*EcoRI*, *NotI*, *XhoI*, *EcoRV*, or *XbaI* of the pENTR vectors).

If you know your nucleic acid molecule of interest does not have, for example, an *XhoI* site, you can make a PCR product that has this structure:

Xho I

```

5' ATG nnn nnn --- nnn TAA ctc gag nnn nnn 3'
3' tac nnn nnn --- nnn att gag ctc nnn nnn 5'

```

After cutting with *Xho*I, the fragment is ready to clone:

```
5' ATG nnn nnn --- nnn TAA c      3'
3' tac nnn nnn --- nnn att gag ct  5'
```

(If you follow this example, don't forget to put a phosphate on the amino oligo.)

Option 2: This PCR product could be cloned into two Entry Vectors to give the desired products, between the *Xmn*I and *Xho*I sites: pENTR1A (Figures 10A, 10B) or pENTR7 (Figures 16A, 16B). If you clone into pENTR1A, amino fusions will have the minimal number of amino acids between the fusion domain and your nucleic acid molecule of interest, but the fusion cannot be removed with TEV protease. The converse is true of clones in pENTR7, i.e., an amino fusion can be cleaved with TEV protease, at the cost of more amino acids between the fusion and your nucleic acid molecule of interest.

In this example, let us choose to clone our hypothetical nucleic acid molecule of interest into pENTR7, between the *Xmn*I and *Xho*I sites. Once this is accomplished, several optional protocols using the Entry Clone pENTR7 may be followed:

Option 3: Since the nucleic acid molecule of interest has been amplified with PCR, it may be desirable to sequence it. To do this, transfer the nucleic acid molecule of interest from the Entry Vector into a vector that has priming sites for the standard sequencing primers. Such a vector is pDEST6 (Figures 26A, 26B). This Destination Vector places the nucleic acid molecule of interest in the opposite orientation to the lac promoter (which is leaky -- see Example 3 above). If the gene product is toxic to *E. coli*, this Destination Vector will minimize its toxicity.

Option 4: While the sequencing is going on, you might wish to check the expression of the nucleic acid molecule of interest in, for example, CHO cells, by recombining the nucleic acid molecule of interest into a CMV promoter vector (pDEST7, Figure 27; or pDEST12, Figure 32), or into a baculovirus vector (pDEST8, Figure 28; or pDEST10, Figure 30) for expression in insect cells. Both

of these vectors will transcribe the coding sequence of your nucleic acid molecule of interest, and translate it from the ATG of the PCR product using the Kozak bases upstream of the *Xmn*I site.

Option 5: If you wish to purify protein, for example to make antibodies, you can clone the nucleic acid molecule of interest into a His6 fusion vector, pDEST2 (Figure 22). Since the nucleic acid molecule of interest is cloned downstream of the TEV protease cleavage domain of pENTR7 (Figure 16), the amino acid sequence of the protein produced will be:

[----- attB1 -----] TEV protease
 NH2- MSYYHHHHHHGITSLYKKAGFFENLYFQ↓GTM----COOH

The attB site and the restriction sites used to make the Destination and Entry Vectors are translated into the underlined 11 amino acids (GITSLYKKAGF). Cleavage with TEV protease (arrow) leaves two amino acids, GT, on the amino end of the gene product.

See Figure 55 for an example of a nucleic acid molecule of interest, the chloramphenicol acetyl transferase (CAT) gene, cloned into pENTR7 (Figure 16) as a blunt (amino)-*Xho*I (carboxy) fragment, then cloned by recombination into the His6 fusion vector pDEST2 (Figure 22).

Option 6: If the His6 fusion protein is insoluble, you may go on and try a GST fusion. The appropriate Destination vector is pDEST3 (Figure 23).

Option 7: If you need to make RNA probes and prefer SP6 RNA polymerase, you can make the top strand RNA with your nucleic acid molecule of interest cloned into pSPORT+ (pDEST5 (Figures 25A, 25B)), and the bottom strand RNA with the nucleic acid molecule of interest cloned into pSPORT(-) (pDEST6 (Figures 26A, 26B)). Opposing promoters for T7 RNA polymerase and SP6 RNA polymerase are also present in these clones.

Option 8: It is often worthwhile to clone your nucleic acid molecule of interest into a variety of Destination Vectors in the same experiment. For example, if the number of colonies varies widely when the various recombination reactions are transformed into *E. coli*, this may be an indication that the nucleic acid molecule of interest is toxic in some contexts. (This problem is more clearly evident when a positive control gene is used for each Destination Vector.) Specifically, if many more colonies are obtained when the nucleic acid molecule of interest is recombined into pDEST6 than in pDEST5, there is a good chance that leakiness of the lac promoter is causing some expression of the nucleic acid molecule of interest in pSPORT "+" (which is not harmful in pDEST6 because the nucleic acid molecule of interest is in the opposite orientation).

Example 16: Demonstration of a One-tube Transfer of a PCR Product (or Expression Clone) to Expression Clone via a Recombinational Cloning Reaction

In the BxP recombination (Entry or Gateway) reaction described herein, a DNA segment flanked by attB1 and attB2 sites in a plasmid conferring ampicillin resistance was transferred by recombination into an attP plasmid conferring kanamycin resistance, which resulted in a product molecule wherein the DNA segment was flanked by attL sites (attL1 and attL2). This product plasmid comprises an "attL Entry Clone" molecule, because it can react with a "attR Destination Vector" molecule via the LxR (Destination) reaction, resulting in the transfer of the DNA segment to a new (ampicillin resistant) vector. In the previously described examples, it was necessary to transform the BxP reaction products into *E. coli*, select kanamycin resistant colonies, grow those colonies in liquid culture, and prepare miniprep DNA, before reacting this DNA with a Destination Vector in an LxR reaction.

The goal of the following experiment was to eliminate the transformation and miniprep DNA steps, by adding the BxP Reaction products directly to an LxR Reaction. This is especially appropriate when the DNA segment flanked by attB sites is a PCR product instead of a plasmid, because the PCR product cannot give

ampicillin-resistant colonies upon transformation, whereas attB plasmids (in general) carry an ampicillin resistance gene. Thus use of a PCR product flanked by attB sites in a BxP Reaction allows one to select for the ampicillin resistance encoded by the desired attB product of a subsequent LxR Reaction.

Two reactions were prepared: Reaction A, negative control, no attB PCR product, (8 μ l) contained 50 ng pEZC7102 (attP Donor plasmid, confers kanamycin resistance) and 2 μ l BxP Clonase (22 ng / μ l Int protein and 8 ng/ μ l IHF protein) in BxP buffer (25 mM Tris HCl, pH 7.8, 70 mM KCl, 5 mM spermidine, 0.5 mM EDTA, 250 μ g / ml BSA). Reaction B (24 μ l) contained 150 ng pEZC7102, 6 μ l BxP Clonase, and 120 ng of the attB -tet-PCR product in the same buffer as reaction A. The attB - tet - PCR product comprised the tetracycline resistance gene of plasmid pBR322, amplified with two primers containing either attB1 or attB2 sites, and having 4 Gs at their 5' ends, as described earlier.

The two reactions were incubated at 25°C for 30 minutes. Then aliquots of these reactions were added to new components that comprised LxR Reactions or appropriate controls for the LxR Reaction. Five new reactions were thus produced:

Reaction 1: 5 μ l of reaction A was added to a 5 μ l LxR Reaction containing 25 ng *Nco*I-cut pEZC8402 (the attR Destination Vector plasmid) in LxR buffer (37.5 mM Tris HCl, pH 7.7, 16.5 mM NaCl, 35 mM KCl, 5 mM spermidine, 375 μ g / ml BSA), and 1 μ l of GATEWAY™ LR Clonase™ Enzyme Mix (total volume of 10 μ l).

Reaction 2: Same as reaction 1, except 5 μ l of reaction B (positive) were added instead of reaction A (negative).

Reaction 3: Same as reaction 2, except that the amounts of *Nco*-cut pEZC8402 and GATEWAY™ LR Clonase™ Enzyme Mix were doubled, to 50 ng and 2 μ l, respectively.

Reaction 4: Same as reaction 2, except that 25 ng of pEZ11104 (a positive control attL Entry Clone plasmid) were added in addition to the aliquot of reaction B.

Reaction 5: Positive control LxR Reaction, containing 25 ng *Nco*I-cut pEYC8402, 25 ng pEZ11104, 37.5 mM Tris HCl pH 7.7, 16.5 mM NaCl, 35 mM KCl, 5 mM spermidine, 375 µg / ml BSA and 1 µl GATEWAY™ LR Clonase™ Enzyme Mix in a total volume of 5 µl.

All five reactions were incubated at 25°C for 30 minutes. Then, 1 µl aliquots of each of the above five reactions, plus 1 µl from the remaining volume of Reaction B, the standard BxP Reaction, were used to transform 50 µl competent DH5α *E. coli*. DNA and cells were incubated on ice for 15 min., heat shocked at 42°C for 45 sec., and 450 µl SOC were added. Each tube was incubated with shaking at 37°C for 60 min. Aliquots of 100 µl and 400 µl of each transformation were plated on LB plates containing either 50 µg/ml kanamycin or 100 µg/ml ampicillin (see Table 2). A transformation with 10 pg of pUC19 DNA (plated on LB-amp₁₀₀) served as a control on the transformation efficiency of the DH5α cells. Following incubation overnight at 37°C, the number of colonies on each plate was determined.

Results of these reactions are shown in Table 2.

Table 2*

Reaction No.	1	2	3	4	5	6
	Number of Colonies					
Vol. plated:	Neg. Control BxP Reaction	1X pEYC8402 and LR Clonase™	2X pEYC8402 and LR Clonase™	LxR Reaction with Pos. Control DNA	LxR Reaction alone	BxP Reaction alone
100 µl	2	1	8	9	~1000	~1000
400 µl	5	10	35	62	>2000	>2000
Selection:	Kan	Amp	Amp	Amp	Amp	Kan

*(Transformation with pUC 19 DNA yielded 1.4×10^9 CFU/µg DNA.)

34 of the 43 colonies obtained from Reaction 3 were picked into 2 ml Terrific Broth with 100 µg/ml ampicillin and these cultures were grown overnight, with shaking, at 37°C. 27 of the 34 cultures gave at least moderate growth, and of these 24 were used to prepare miniprep DNA, using the standard protocol. These 24 DNAs were initially analyzed as supercoiled (SC) DNA on a 1% agarose gel to identify those with inserts and to estimate the sizes of the inserts. Fifteen of the 24 samples displayed SC DNA of the size predicted (5553 bp) if **tetx7102** had correctly recombined with **pEJC8402** to yield **tetx8402**. One of these samples contained two plasmids, one of ~5500 bp and a one of ~3500 bp. The majority of the remaining clones were approximately 4100 bp in size.

All 15 of the clones displaying SC DNA of predicted size (~5500 bp) were analyzed by two different double digests with restriction endonucleases to confirm the structure of the expected product: **tetx8402**. (See plasmid maps, Figures 57-59) In one set of digests, the DNAs were treated with Not I and Eco RI, which should cut the predicted product just outside both attB sites, releasing the tet^r insert on a fragment of 1475 bp. In the second set of digests, the DNAs were digested with *NotI* and with *NruI*. *NruI* cleaves asymmetrically within the subcloned tet^r insert, and together with *NotI* will release a fragment of 1019 bp.

Of the 15 clones analyzed by double restriction digestion, 14 revealed the predicted sizes of fragments for the expected product.

Interpretation:

The DNA components of Reaction B, pEJC7102 and attB-tet-PCR, are shown in Figure 56. The desired product of BxP Reaction B is tetx7102, depicted in Figure 57. The LxR Reaction recombines the product of the BxP Reaction, tetx7102 (Figure 57), with the Destination Vector, pEJC8402, shown in Figure 58. The LxR Reaction with tetx7102 plus pEJC8402 is predicted to yield the desired product tetx8402, shown in Figure 59.

Reaction 2, which combined the BxP Reaction and LxR Reaction, gave few colonies beyond those of the negative control Reaction. In contrast, Reaction 3, with twice the amount of pEJC8402 (Figure 58) and LxR Clonase, yielded a

larger number of colonies. These colonies were analyzed further, by restriction digestion, to confirm the presence of expected product. Reaction 4 included a known amount of attL Entry Clone plasmid in the combined BxP-plus-LxR reaction. But reaction 4 yielded only about 1% of the colonies obtained when the same DNA was used in a LxR reaction alone, Reaction 6. This result suggests that the LxR reaction may be inhibited by components of the BxP reaction.

Restriction endonuclease analysis of the products of Reaction 3 revealed that a sizeable proportion of the colonies (14 of the 34 analyzed) contained the desired *tet^r* subclone, *tetx8402* (Figure 59).

The above results establish the feasibility of performing first a BxP recombination reaction followed by a LxR recombination reaction -- in the same tube -- simply by adding the appropriate buffer mix, recombination proteins, and DNAs to a completed BxP reaction. This method should prove useful as a faster method to convert attB-containing PCR products into different Expression Clones, eliminating the need to isolate first the intermediate attL-PCR insert subclones, before recombining these with Destination Vectors. This may prove especially valuable for automated applications of these reactions.

This same one-tube approach allows for the rapid transfer of nucleic acid molecules contained in attB plasmid clones into new functional vectors as well. As in the above examples, attL subclones generated in a BxP Reaction can be recombined directly with various Destination Vectors in a LxR reaction. The only additional requirement for using attB plasmids, instead of attB-containing PCR products, is that the Destination Vector(s) employed must contain a different selection marker from the one present on the attB plasmid itself and the attP vector.

Two alternative protocols for a one-tube reaction have also proven useful and somewhat more optimal than the conditions described above.

Alternative 1:

Reaction buffer contained 50 mM Tris-HCl (pH 7.5), 50 mM NaCl, 0.25 mM EDTA, 2.5 mM spermidine, and 200 µg/ml BSA. After a 16 (or 3) hour incubation of the PCR product (100 ng) + attP Donor plasmid (100 ng) +

GATEWAY™ BP Clonase™ Enzyme Mix + Destination Vector (100 ng), 2 µl of GATEWAY™ LR Clonase™ Enzyme Mix (per 10 µl reaction mix) was added and the mixture was incubated an additional 6 (or 2) hours at 25°C. Stop solution was then added as above and the mixture was incubated at 37°C as above and transformed by electroporation with 1 µl directly into electrocompetent host cells. Results of this series of experiments demonstrated that longer incubation times (16 hours vs. 3 hours for the BP Reaction, 6 hours vs. 2 hours for the LR Reaction) resulted in about twice as many colonies being obtained as for the shorter incubation times. With two independent genes, 10/10 colonies having the correct cloning patterns were obtained.

Alternative 2:

A standard BP Reaction under the reaction conditions described above for Alternative 1 was performed for 2 hours at 25°C. Following the BP Reaction, the following components were added to the reaction mixture in a total volume of 7 µl:

20 mM Tris-HCl, pH 7.5
100 mM NaCl
5 µg/ml Xis-His6
15% glycerol
~1000 ng of Destination Vector

The reaction mixture was then incubated for 2 hours at 25°C, and 2.5 µl of stop solution (containing 2 µg/ml proteinase K) was added and the mixture was incubated at 37°C for an additional 10 minutes. Chemically competent host cells were then transformed with 2 µl of the reaction mixture, or electrocompetent host cells (e.g., EMax DH10B cells; Life Technologies, Inc.) were electroporated with 2 µl of the reaction mixture per 25-40 µl of cells. Following transformation, mixtures were diluted with SOC, incubated at 37°C, and plated as described above on media selecting for the selection markers on the Destination Vector and the Entry clone (B x P reaction product). Analogous results to those described for Alternative 1 were obtained with these reaction conditions -- a higher level of colonies containing correctly recombined reaction products were observed.

Example 17: Demonstration of a One-tube Transfer of a PCR Product (or Expression Clone) to Expression Clone via a Recombinational Cloning Reaction

5 Single-tube transfer of PCR product DNA or Expression Clones into Expression Clones by recombinational cloning has also been accomplished using a procedure modified from that described in Example 16. This procedure is as follows:

10 •Perform a standard BP (Gateway) Reaction (see Examples 9 and 10) in 20 µl volume at 25°C for 1 hour.

15 •After the incubation is over, take a 10 µl aliquot from the 20 µl total volume and add 1 µl of Proteinase K (2 mg/ml) and incubate at 37°C for 10 minutes. This first aliquot can be used for transformation and gel assay of BP reaction analysis. Plate BP reaction transformation on LB plates with **Kanamycin** (50 µg/ml).

20 •Add the following reagents to the remaining 10 µl aliquot of the BP reaction:

 1 µl of 0.75 M NaCl

 2 µl of destination vector (150 ng/µl)

 4 µl of LR Clonase™ (after thawing and brief mixing)

25 •Mix all reagents well and incubate at 25°C for 3 hours. Stop the reaction at the end of incubation with 1.7 µl of Proteinase K (2 mg/ml) and incubate at 37°C for 10 minutes.

30 •Transform 2 µl of the completed reaction into 100 µl of competent cells. Plate 100 µl and 400 µl on LB plates with **Ampicillin** (100 µg/ml).

Notes:

•If your competent cells are less than 10⁸ CFU/µg, and you are concerned about getting enough colonies, you can improve the yield several fold by incubating the

BP reaction for 6-20 hours. Electroporation also can yield better colony output than chemical transformation.

•PCR products greater than about 5-6 kb show significantly lower cloning efficiency in the BP reaction. In this case, we recommend using longer incubation times for both BP and LR steps.

•If you want to move your insert gene into several destination vectors simultaneously, then scale up the initial BP reaction volume so that you have a 10 µl aliquot for adding each destination vector.

Example 18: Optimization of GATEWAY™ Clonase™ Enzyme Compositions

The enzyme compositions containing Int and IHF (for BP Reactions) were optimized using a standard functional recombinational cloning reaction (a BP reaction) between attB-containing plasmids and attP-containing plasmids, according to the following protocol:

Materials and Methods:

Substrates:

AttP - supercoiled pDONR201

AttB - linear ~ 1Kb [³H]PCR product amplified from pEZC7501

Proteins:

IntH6 -- His₆-carboxy- tagged λ Integrase

IHF -- Integration Host Factor

Clonase:

50 ng/µl IntH6 and 20 ng/µl IHF, admixed in 25 mM Tris- HCl (pH 7.5), 22 mM NaCl, 5 mM EDTA, 1 mg/ml BSA, 5 mM Spermidine, and 50% glycerol.

-140-

Reaction Mixture (total volume of 40 μ l):

1000 ng AttP plasmid

600 ng AttB [3 H] PCR product

8 μ l Clonase (400 ng IntH6, 160 ng IHF) in 25 mM Tris-HCl (pH 7.5),
22 mM NaCl, 5 mM EDTA, 1 mg/ml BSA, 5 mM Spermidine, 5 mM
DTT.

Reaction mixture was incubated for 1 hour at 25°C, 4 μ l of 2 μ g/ μ l
proteinase K was added and mixture was incubated for an additional 20 minutes
at 37°C. Mixture was then extracted with an equal volume of Phenol/Chloroform/
Isoamyl alcohol. The aqueous layer was then collected, and 0.1 volumes of 3 M
sodium acetate and 2 volumes of cold 100% ethanol were added. Tubes were
then spun in a microcentrifuge at maximum RPM for 10 minutes at room
temperature. Ethanol was decanted, and pellets were rinsed with 70% ethanol and
re-centrifuged as above. Ethanol was decanted, and pellets were allowed to air
dry for 5-10 minutes and then dissolved in 20 μ l of 33 mM Tris-Acetate (pH 7.8),
66 mM potassium acetate, 10 mM magnesium acetate, 1 mM DTT, and 1mM
ATP. 2 units of exonuclease V (e.g., Plasmid Safe; EpiCentre, Inc., Madison, WI)
was then added, and the mixture was incubated at 37°C for 30 minutes.

Samples were then TCA-washed by spotting 30 μ l of reaction mixture
onto a Whatman GF/C filter, washing filters once with 10% TCA + 1% NaPPi for
10 minutes, three times with 5% TCA for 5 minutes each, and twice with ethanol
for 5 minutes each. Filters were then dried under a heat lamp, placed into a
scintillation vial, and counted on a β liquid scintillation counter (LSC).

The principle behind this assay is that, after exonuclease V digestion, only
double-stranded circular DNA survives in an acid-insoluble form. All DNA
substrates and products that have free ends are digested to an acid-soluble form
and are not retained on the filters. Therefore, only the 3 H-labeled attB linear DNA
which ends up in circular form after both inter- and intramolecular integration is
complete is resistant to digestion and is recovered as acid-insoluble product.
Optimal enzyme and buffer formulations in the Clonase compositions therefore are
those that give the highest levels of circularized 3 H-labeled attB-containing

sequences, as determined by highest cpm in the LSC. Although this assay was designed for optimization of GATEWAY™ BP Clonase™ Enzyme Mix compositions (Int + IHF), the same type of assay may be performed to optimize GATEWAY™ LR Clonase™ Enzyme Mix compositions (Int + IHF + Xis), except that the reaction mixtures would comprise 1000 ng of AttR (instead of AttP) and 600 ng of AttL (instead of AttB), and 40 ng of His₆-carboxy- tagged Xis (XisH6) in addition to the IntH6 and IHF.

Example 19: Testing Functionality of Entry and Destination Vectors

As part of assessment of the functionality of particular vectors of the invention, it is important to functionally test the ability of the vectors to recombine. This assessment can be carried out by performing a recombinational cloning reaction (as schematized in Figures 2, 4, and 5A and 5B, and as described herein and in commonly owned U.S. Application Nos. 08/486,139, filed June 7, 1995, 08/663,002, filed June 7, 1996 (now U.S. Patent No. 5,888,732), 09/005,476, filed January 12, 1998, and 09/177,387, filed October 23, 1998, the disclosures of all of which are incorporated by reference herein in their entireties), by transforming E. coli and scoring colony forming units. However, an alternative assay may also be performed to allow faster, more simple assessment of the functionality of a given Entry or Destination Vector by agarose gel electrophoresis. The following is a description of such an in vitro assay.

Materials and Methods:

Plasmid templates pEZC1301 (Figure 84) and pEZC1313 (Figure 85), each containing a single wild type att site, were used for the generation of PCR products containing attL or attR sites, respectively. Plasmid templates were linearized with *A*la¹NI, phenol extracted, ethanol precipitated and dissolved in TE to a concentration of 1 ng/μl.

PCR primers (capital letters represent base changes from wildtype):

attL1 gggg agcct gctttttGtacAaa gttggcatta taaaaaagca ttgc
attL2 gggg agcct gctttCttGtacAaa gttggcatta taaaaaagca ttgc
attL right tgttgccggg aagctagagt aa

attR1 gggg Acaag ttTgtaCaaaaaagc tgaacgaga aacgtaaaat
attR2 gggg Acaag ttTgtaCaaGaaagc tgaacgaga aacgtaaaat
attR right ca gacggcatga tgaacctgaa

5

10

PCR primers were dissolved in TE to a concentration of 500 pmol/ μ l. Primer mixes were prepared, consisting of attL1 + attLright primers, attL2 + attLright primers, attR1 + attRright primers, and attR2 + attRright primers, each mix containing 20 pmol/ μ l of each primer.

PCR reactions:

15

1 μ l plasmid template (1 ng)
1 μ l primer pairs (20 pmoles of each)
3 μ l of H₂O
45 μ l of Platinum PCR SuperMix® (Life Technologies, Inc.)

20

Cycling conditions (performed in MJ thermocycler):

95°C/2 minutes
94°C/30 seconds
25 cycles of 58°C/30 seconds and 72°C/1.5 minutes
72°C/5 minutes
5°C/hold

25

The resulting attL PCR product was 1.5 kb, and the resulting attR PCR product was 1.0 kb.

30

PCR reactions were PEG/MgCl₂ precipitated by adding 150 μ l H₂O and 100 μ l of 3x PEG/ MgCl₂ solution followed by centrifugation. The PCR products were dissolved in 50 μ l of TE. Quantification of the PCR product was performed by gel electrophoresis of 1 μ l and was estimated to be 50-100 ng/ μ l.

Recombination reactions of PCR products containing attL or attR sites with GATEWAY™ plasmids was performed as follows:

8 µl of H₂O

2 µl of attL or attR PCR product (100-200 ng)

2 µl of GATEWAY™ plasmid (100 ng)

4 µl of 5x Destination buffer

4 µl of GATEWAY™ LR Clonase™ Enzyme Mix

20 µl total volume (the reactions can be scaled down to a 5 µl total volume by adjusting the volumes of the components to about ¼ of those shown above, while keeping the stoichiometries the same).

Clonase reactions were incubated at 25°C for 2 hours. 2 µl of proteinase K (2 mg/ml) was added to stop the reaction. 10 µl was then run on a 1 % agarose gel. Positive control reactions were performed by reacting attL1 PCR product (1.0 kb) with attR1 PCR product (1.5 kb) and by similarly reacting attL2 PCR product with attR2 PCR product to observe the formation of a larger (2.5 kb) recombination product. Negative controls were similarly performed by reacting attL1 PCR product with attR2 PCR product and vice versa or reactions of attL PCR product with an attL plasmid, etc.

In alternative assays, to test attB Entry vectors, plasmids containing single attP sites were used. Plasmids containing single att sites could also be used as recombination substrates in general to test all Entry and Destination vectors (*i.e.*, those containing attL, attR, attB and attP sites). This would eliminate the need to do PCR reactions.

Results:

Destination and Entry plasmids when reacted with appropriate att-containing PCR products formed linear recombinant molecules that could be easily visualized on an agarose gel when compared to control reactions containing no attL or attR PCR product. Thus, the functionality of Destination and Entry vectors constructed according to the invention may be determined either by carrying out the Destination or Entry recombination reactions as depicted in

Figures 2, 4, and 5A and 5B, or more rapidly by carrying out the linearization assay described in this Example.

Example 20: PCR Cloning Using Universal Adapter-Primers

As described herein, the cloning of PCR products using the GATEWAY™ PCR Cloning System (Life Technologies, Inc.; Rockville, MD) requires the addition of attB sites (attB1 and attB2) to the ends of gene-specific primers used in the PCR reaction. The protocols described in the preceding Examples suggest that the user add 29 bp (25 bp containing the attB site plus four G residues) to the gene-specific primer. It would be advantageous to high volume users of the GATEWAY™ PCR Cloning System to generate attB-containing PCR product using universal attB adapter-primers in combination with shorter gene-specific primers containing a specified overlap to the adapters. The following experiments demonstrate the utility of this strategy using universal attB adapter-primers and gene-specific primers containing overlaps of various lengths from 6 bp to 18 bp. The results demonstrate that gene-specific primers with overlaps of 10 bp to 18 bp can be used successfully in PCR amplifications with universal attB adapter-primers to generate full-length PCR products. These PCR products can then be successfully cloned with high fidelity in a specified orientation using the GATEWAY™ PCR Cloning System.

Methods and Results:

To demonstrate that universal attB adapter-primers can be used with gene-specific primers containing partial attB sites in PCR reactions to generate full-length PCR product, a small 256 bp region of the human hemoglobin cDNA was chosen as a target so that intermediate sized products could be distinguished from full-length products by agarose gel electrophoresis.

The following oligonucleotides were used:

B1-Hgb: GGGG ACA AGT TTG TAC AAA AAA GCA GGC T-5'-Hgb*
B2-Hgb: GGGG ACC ACT TTG TAC AAG AAA GCT GGG T-3'-Hgb**

-145-

18B1-Hgb: TG TAC AAA AAA GCA GGC T-5'-Hgb
 18B2-Hgb: TG TAC AAG AAA GCT GGG T-3'-Hgb
 15B1-Hgb: AC AAA AAA GCA GGC T-5'-Hgb
 15B2-Hgb: AC AAG AAA GCT GGG T-3'-Hgb
 5 12B1-Hgb: AA AAA GCA GGC T-5'-Hgb
 12B2-Hgb: AG AAA GCT GGG T-3'-Hgb
 11B1-Hgb: A AAA GCA GGC T-5'-Hgb
 11B2-Hgb: G AAA GCT GGG T-3'-Hgb
 10B1-Hgb: AAA GCA GGC T-5'-Hgb
 10 10B2-Hgb: AAA GCT GGG T-3'-Hgb
 9B1-Hgb: AA GCA GGC T-5'-Hgb
 9B2-Hgb: AA GCT GGG T-3'-Hgb
 8B1-Hgb: A GCA GGC T-5'-Hgb
 8B2-Hgb: A GCT GGG T-3'-Hgb
 15 7B1-Hgb: GCA GGC T-5'-Hgb
 7B2-Hgb: GCT GGG T-3'-Hgb
 6B1-Hgb: CA GGC T-5'-Hgb
 6B2-Hgb: CT GGG T-3'-Hgb

 20 attB1 adapter: GGGG ACA AGT TTG TAC AAA AAA GCA GGC T
 attB2 adapter: GGGG ACC ACT TTG TAC AAG AAA GCT GGG T

 * -5'-Hgb = GTC ACT AGC CTG TGG AGC AAG A
 ** -3'-Hgb = AGG ATG GCA GAG GGA GAC GAC A

25

30

The aim of these experiments was to develop a simple and efficient universal adapter PCR method to generate attB containing PCR products suitable for use in the GATEWAY™ PCR Cloning System. The reaction mixtures and thermocycling conditions should be simple and efficient so that the universal adapter PCR method could be routinely applicable to any PCR product cloning application.

35

PCR reaction conditions were initially found that could successfully amplify predominately full-length PCR product using gene-specific primers containing 18bp and 15 bp overlap with universal attB primers. These conditions are outlined below:

-146-

10 pmoles of gene-specific primers
10 pmoles of universal attB adapter-primers
1 ng of plasmid containing the human hemoglobin cDNA.
100 ng of human leukocyte cDNA library DNA.
5 μ l of 10x PLATINUM Taq HiFi® reaction buffer (Life Technologies, Inc.)
2 μ l of 50 mM MgSO₄
1 μ l of 10 mM dNTPs
0.2 μ l of PLATINUM Taq HiFi® (1.0 unit)
H₂O to 50 μ l total reaction volume

Cycling conditions:

25 x

95°C/5 min
94°C/15 sec
50°C/30 sec
68°C/1 min
68°C/5 min
5°C/hold

To assess the efficiency of the method, 2 μ l (1/25) of the 50 μ l PCR reaction was electrophoresed in a 3 % Agarose-1000 gel. With overlaps of 12 bp or less, smaller intermediate products containing one or no universal attB adapter predominated the reactions. Further optimization of PCR reaction conditions was obtained by titrating the amounts of gene-specific primers and universal attB adapter-primers. The PCR reactions were set up as outlined above except that the amounts of primers added were:

0, 1, 3 or 10 pmoles of gene-specific primers
0, 10, 30 or 100 pmoles of adapter-primers

Cycling conditions:

25 x		95°C/3 min
		94°C/15 sec
		50°C/45 sec
		68°C/1 min
		68°C/5 min
		5°C/hold

The use of limiting amounts of gene-specific primers (3 pmoles) and excess adapter-primers (30 pmoles) reduced the amounts of smaller intermediate products. Using these reaction conditions the overlap necessary to obtain predominately full-length PCR product was reduced to 12 bp. The amounts of gene-specific and adapter-primers was further optimized in the following PCR reactions:

0, 1, 2 or 3 pmoles of gene-specific primers
0, 30, 40 or 50 pmoles of adapter-primers

Cycling conditions:

25 x

95°C/3 min
94°C/15 sec
48°C/1 min
68°C/1 min
68°C/5 min
5°C/hold

The use of 2 pmoles of gene-specific primers and 40 pmoles of adapter-primers further reduced the amounts of intermediate products and generated predominately full-length PCR products with gene-specific primers containing an 11 bp overlap. The success of the PCR reactions can be assessed in any PCR application by performing a no adapter control. The use of limiting amounts of gene-specific primers should give faint or barely visible bands when 1/25 to 1/10 of the PCR reaction is electrophoresed on a standard agarose gel. Addition of the

universal attB adapter-primers should generate a robust PCR reaction with a much higher overall yield of product.

PCR products from reactions using the 18 bp, 15 bp, 12 bp, 11 bp and 10 bp overlap gene-specific primers were purified using the CONCERT® Rapid PCR Purification System (PCR products greater than 500 bp can be PEG precipitated). The purified PCR products were subsequently cloned into an attP containing plasmid vector using the GATEWAY™ PCR Cloning System (Life Technologies, Inc.; Rockville, MD) and transformed into *E. coli*. Colonies were selected and counted on the appropriate antibiotic media and screened by PCR for correct inserts and orientation.

Raw PCR products (unpurified) from the attB adapter PCR of a plasmid clone of part of the human beta-globin (Hgb) gene were also used in GATEWAY™ PCR Cloning System reactions. PCR products generated with the full attB B1/B2-Hgb, the 12B1/B2, 11B1/B2 and 10B1/B2 attB overlap Hgb primers were successfully cloned into the GATEWAY™ pENTR21 attP vector (Figure 49). 24 colonies from each (24 x 4 = 96 total) were tested and each was verified by PCR to contain correct inserts. The cloning efficiency expressed as cfu/ml is shown below:

Primer Used	cfu/ml
Hgb full attB	8,700
Hgb 12 bp overlap	21,000
Hgb 11 bp overlap	20,500
Hgb 10 bp overlap	13,500
GFP control	1,300

Interestingly, the overlap PCR products cloned with higher efficiency than did the full attB PCR product. Presumably, and as verified by visualization on agarose gel, the adapter PCR products were slightly cleaner than was the full attB PCR product. The differences in colony output may also reflect the proportion of PCR product molecules with intact attB sites.

Using the attB adapter PCR method, PCR primers with 12 bp attB overlaps were used to amplify cDNAs of different sizes (ranging from 1 to 4 kb)

from a leukocyte cDNA library and from first strand cDNA prepared from HeLa total RNA. While three of the four cDNAs were able to be amplified by this method, a non-specific amplification product was also observed that under some conditions would interfere with the gene-specific amplification. This non-specific product was amplified in reactions containing the attB adapter-primers alone without any gene-specific overlap primers present. The non-specific amplification product was reduced by increasing the stringency of the PCR reaction and lowering the attB adapter PCR primer concentration.

These results indicate that the adapter-primer PCR approach described in this Example will work well for cloned genes. These results also demonstrate the development of a simple and efficient method to amplify PCR products that are compatible with the GATEWAY™ PCR Cloning System that allows the use of shorter gene-specific primers that partially overlap universal attB adapter-primers. In routine PCR cloning applications, the use of 12 bp overlaps is recommended. The methods described in this Example can thus reduce the length of gene-specific primers by up to 17 residues or more, resulting in a significant savings in oligonucleotide costs for high volume users of the GATEWAY™ PCR Cloning System. In addition, using the methods and assays described in this Example, one of ordinary skill can, using only routine experimentation, design and use analogous primer-adapters based on or containing other recombination sites or fragments thereof, such as *attL*, *attR*, *attP*, *lox*, FRT, etc.

Example 21: Mutational Analysis of the Bacteriophage Lambda attL and attR Sites: Determinants of att Site Specificity in Site-specific Recombination

To investigate the determinants of *att* site specificity, the bacteriophage lambda *attL* and *attR* sites were systematically mutagenized. As noted herein, the determinants of specificity have previously been localized to the 7 bp overlap region (TTTATAC, which is defined by the cut sites for the integrase protein and is the region where strand exchange takes place) within the 15 bp core region (GCTTTTTTATACTAA) which is identical in all four lambda *att* sites, *attB*, *attP*, *attL* and *attR*. This core region, however, has not heretofore been systematically

mutagenized and examined to define precisely which mutations produce unique changes in *att* site specificity.

Therefore, to examine the effect of *att* sequence on site specificity, mutant *attL* and *attR* sites were generated by PCR and tested in an *in vitro* site-specific recombination assay. In this way all possible single base pair changes within the 7 bp overlap region of the core *att* site were generated as well as five additional changes outside the 7 bp overlap but within the 15 bp core *att* site. Each *attL* PCR substrate was tested in the *in vitro* recombination assay with each of the *attR* PCR substrates.

Methods

To examine both the efficiency and specificity of recombination of mutant *attL* and *attR* sites, a simple *in vitro* site-specific recombination assay was developed. Since the core regions of *attL* and *attR* lie near the ends of these sites, it was possible to incorporate the desired nucleotide base changes within PCR primers and generate a series of PCR products containing mutant *attL* and *attR* sites. PCR products containing *attL* and *attR* sites were used as substrates in an *in vitro* reaction with GATEWAY™ LR Clonase™ Enzyme Mix (Life Technologies, Inc.; Rockville, MD). Recombination between a 1.5 kb *attL* PCR product and a 1.0 kb *attR* PCR product resulted in a 2.5 kb recombinant molecule that was monitored using agarose gel electrophoresis and ethidium bromide staining.

Plasmid templates pEZC1301 (Figure 84) and pEZC1313 (Figure 85), each containing a single wild type *attL* or *attR* site, respectively, were used for the generation of recombination substrates. The following list shows primers that were used in PCR reactions to generate the *attL* PCR products that were used as substrates in L x R Clonase reactions (capital letters represent changes from the wild-type sequence, and the underline represents the 7 bp overlap region within the 15 bp core *att* site; a similar set of PCR primers was used to prepare the *attR* PCR products containing matching mutations):

-151-

GATEWAY™ sites (note: attL2 sequence in GATEWAY™ plasmids begins "accca" while the attL2 site in this example begins "agcct" to reflect wild-type attL outside the core region.):

5

attL1: gggg agcct gcttttttGtacAaa gttggcatta taaaaa-
agca ttgc

10

attL2: gggg agcct gcttttCttGtacAaa gttggcatta taaaaa-
agca ttgc

Wild-type:

15

attL0: gggg agcct gctttttttatactaa gttggcatta taaaaa-
agca ttgc

Single base changes from wild-type:

attLT1A: gggg agcct gcttttAttatactaa gttggcatta taaaaa-
agca ttgc

20

attLT1C: gggg agcct gcttttCttatactaa gttggcatta taaaaa-
agca ttgc

attLT1G: gggg agcct gcttttGttatactaa gttggcatta taaaaa-
agca ttgc

25

attLT2A: gggg agcct gcttttAtatactaa gttggcatta taaaaa-
agca ttgc

30

attLT2C: gggg agcct gcttttCtatactaa gttggcatta taaaaa-
agca ttgc

attLT2G: gggg agcct gcttttGtatactaa gttggcatta taaaa-
aagca ttgc

35

-152-

attLT3A: gggg agcct gcttttttAatactaa gttggcatta taaaa-
aagca ttgc

5 attLT3C: gggg agcct gcttttttCatactaa gttggcatta taaaa-
aagca ttgc

10 attLT3G: gggg agcct gcttttttGatactaa gttggcatta taaaa-
aagca ttgc

15 attLA4C: gggg agcct gcttttttCtactaa gttggcatta taaaa-
aagca ttgc

attLA4G: gggg agcct gcttttttGtactaa gttggcatta taaaa-
aagca ttgc

20 attLA4T: gggg agcct gcttttttTtactaa gttggcatta taaaa-
aagca ttgc

25 attLT5A: gggg agcct gcttttttAaactaa gttggcatta taaaa-
aagca ttgc

attLT5C: gggg agcct gcttttttCaactaa gttggcatta taaaa-
aagca ttgc

30 attLT5G: gggg agcct gcttttttGaactaa gttggcatta taaaa-
aagca ttgc

35 attLA6C: gggg agcct gcttttttatCctaa gttggcatta taaaa-
aagca ttgc

-153-

attLA6G: gggg agcct gcttttttatGctaa gttggcatta taaaa-
aagca ttgc

5 attLA6T: gggg agcct gcttttttatTctaa gttggcatta taaaa-
aagca ttgc

10 attLC7A: gggg agcct gcttttttataAtaa gttggcatta taaaa-
aagca ttgc

15 attLC7G: gggg agcct gcttttttataGtaa gttggcatta taaaa-
aagca ttgc

attLC7T: gggg agcct gcttttttataTtaa gttggcatta taaaa-
aagca ttgc

Single base changes outside of the 7 bp overlap:

20 attL8: gggg agcct Acttttttataactaa gttggcatta taaaa-
aagca ttgc

25 attL9: gggg agcct gcCtttttataactaa gttggcatta taaaaa-
agca ttgc

attL10: gggg agcct gcttCtttataactaa gttggcatta taaaaa-
agca ttgc

30 attL14: gggg agcct gcttttttataacCaa gttggcatta taaaaa-
agca ttgc

35 attL15: gggg agcct gcttttttataactaG gttggcatta taaaaa-
agca ttgc

Note: additional vectors wherein the first nine bases are gggg agcca (*i.e.*, substituting an adenine for the thymine in the position immediately preceding the 15-bp core region), which may or may not contain the single base pair substitutions (or deletions) outlined above, can also be used in these experiments.

Recombination reactions of *attL*- and *attR*-containing PCR products was performed as follows:

8 µl of H₂O
2 µl of *attL* PCR product (100 ng)
2 µl of *attR* PCR product (100 ng)
4 µl of 5x buffer
4 µl of GATEWAY™ LR Clonase™ Enzyme Mix
20 µl total volume

Clonase reactions were incubated at 25°C for 2 hours.

2 µl of 10X Clonase stop solution (proteinase K, 2 mg/ml) were added to stop the reaction.

10 µl were run on a 1 % agarose gel.

Results

Each *attL* PCR substrate was tested in the *in vitro* recombination assay with each of the *attR* PCR substrates. Changes within the first three positions of the 7 bp overlap (TTTATAC) strongly altered the specificity of recombination. These mutant *att* sites each recombined as well as the wild-type, but only with their cognate partner mutant; they did not recombine detectably with any other *att* site mutant. In contrast, changes in the last four positions (TTTATAC) only partially altered specificity; these mutants recombined with their cognate mutant as well as wild-type *att* sites and recombined partially with all other mutant *att* sites except for those having mutations in the first three positions of the 7 bp

overlap. Changes outside of the 7 bp overlap were found not to affect specificity of recombination, but some did influence the efficiency of recombination.

Based on these results, the following rules for *att* site specificity were determined:

- Only changes within the 7 bp overlap affect specificity.
- Changes within the first 3 positions strongly affect specificity.
- Changes within the last 4 positions weakly affect specificity.

Mutations that affected the overall efficiency of the recombination reaction were also assessed by this method. In these experiments, a slightly increased (less than 2-fold) recombination efficiency with *attLT1A* and *attLC7T* substrates was observed when these substrates were reacted with their cognate *attR* partners. Also observed were mutations that decreased recombination efficiency (approximately 2-3 fold), including *attLA6G*, *attL14* and *attL15*. These mutations presumably reflect changes that affect Int protein binding at the core *att* site.

The results of these experiments demonstrate that changes within the first three positions of the 7 bp overlap (TTTATAC) strongly altered the specificity of recombination (*i.e.*, *att* sequences with one or more mutations in the first three thymidines would only recombine with their cognate partners and would not cross-react with any other *att* site mutation). In contrast, mutations in the last four positions (TTTATAC) only partially altered specificity (*i.e.*, *att* sequences with one or more mutations in the last four base positions would cross-react partially with the wild-type *att* site and all other mutant *att* sites, except for those having mutations in one or more of the first three positions of the 7 bp overlap). Mutations outside of the 7 bp overlap were not found to affect specificity of recombination, but some were found to influence (*i.e.*, to cause a decrease in) the efficiency of recombination.

Example 22: Discovery of Att Site Mutations That Increase the Cloning Efficiency of GATEWAY™ Cloning Reactions

In experiments designed to understand the determinants of *att* site specificity, point mutations in the core region of *attL* were made. Nucleic acid molecules containing these mutated *attL* sequences were then reacted in an LR

reaction with nucleic acid molecules containing the cognate *attR* site (*i.e.*, an *attR* site containing a mutation corresponding to that in the *attL* site), and recombinational efficiency was determined as described above. Several mutations located in the core region of the att site were noted that either slightly increased (less than 2-fold) or decreased (between 2-4-fold) the efficiency of the recombination reaction (Table 3).

Table 3. *Effects of attL mutations on Recombination Reactions.*

<u>Site</u>	<u>Sequence</u>	<u>Effect on Recombination</u>
attL0	agcctgcttttttataactaagttggcatta	
attL5	agcctgcttttAttataactaagttggcatta	slightly increased
attL6	agcctgctttttttataTtaagttggcatta	slightly increased
attL13	agcctgctttttttatGctaagttggcatta	decreased
attL14	agcctgctttttttatacCaagttggcatta	decreased
attL15	agcctgctttttttataactaGgttggcatta	decreased
consensus	CAACTTnnTnnnAnnAAGTTG	

It was also noted that these mutations presumably reflected changes that either increased or decreased, respectively, the relative affinity of the integrase protein for binding the core att site. A consensus sequence for an integrase core-binding site (CAACTTNNT) has been inferred in the literature but not directly tested (see, *e.g.*, Ross and Landy, *Cell* 33:261-272 (1983)). This consensus core integrase-binding sequence was established by comparing the sequences of each of the four core att sites found in attP and attB as well as the sequences of five non-att sites that resemble the core sequence and to which integrase has been shown to bind in vitro. These experiments suggest that many more att site mutations might be identified which increase the binding of integrase to the core att site and thus increase the efficiency of GATEWAY™ cloning reactions.

Example 23: Effects of Core Region Mutations on Recombination Efficiency

To directly compare the cloning efficiency of mutations in the att site core region, single base changes were made in the attB2 site of an attB1-TET-attB2 PCR product. Nucleic acid molecules containing these mutated attB2 sequences were then reacted in a BP reaction with nucleic acid molecules containing non-cognate attP sites (i.e., wildtype attP2), and recombinational efficiency was determined as described above. The cloning efficiency of these mutant attB2 containing PCR products compared to standard attB1-TET-attB2 PCR product are shown in Table 4.

Table 4. Efficiency of Recombination With Mutated attB2 Sites.

<u>Site</u>	<u>Sequence</u>	<u>Mutation</u>	<u>Cloning Efficiency</u>
attB0	tcaagttagttataaaaaagcaggct		
attB1	ggggacaagtttgtacaaaaagcaggct		
attB2	ggggaccactttgtacaagaaagctgggt		100%
attB2.1	ggggAaactttgtacaagaaagctgggt	C→A	40%
attB2.2	ggggacAactttgtacaagaaagctgggt	C→A	131%
attB2.3	ggggaccCctttgtacaagaaagctgggt	A→C	4%
attB2.4	ggggaccaAttgtacaagaaagctgggt	C→A	11%
attB2.5	ggggaccacGttgtacaagaaagctgggt	T→G	4%
attB2.6	ggggaccactGtgtacaagaaagctgggt	T→G	6%
attB2.7	ggggaccacttGgtacaagaaagctgggt	T→G	1%
attB2.8	ggggaccactttTtacaagaaagctgggt	G→T	0.5%

As noted above, a single base change in the attB2.2 site increased the cloning efficiency of the attB1-TET-attB2.2 PCR product to 131% compared to the attB1-TET-attB2 PCR product. Interestingly, this mutation changes the integrase core binding site of attB2 to a sequence that matches more closely the proposed consensus sequence.

Additional experiments were performed to directly compare the cloning efficiency of an attB1-TET-attB2 PCR product with a PCR product that contained attB sites containing the proposed consensus sequence (*see* Example 22) of an integrase core binding site. The following attB sites were used to amplify attB-TET PCR products:

```
attB1      ggggacaagtttgtacaaaaaagcaggct
attB1.6    ggggacaaCtttgtacaaaaaagTTggct
attB2      ggggaccactttgtacaagaaagctgggt
attB2.10   ggggacAactttgtacaagaaagTtgggt
```

BP reactions were carried out between 300 ng (100 fmoles) of pDONR201 (Figure 49A) with 80 ng (80 fmoles) of attB-TET PCR product in a 20 µl volume with incubation for 1.5 hrs at 25°C, creating pENTR201-TET Entry clones. A comparison of the cloning efficiencies of the above-noted attB sites in BP reactions is shown in Table 5.

Table 5. Cloning efficiency of BP Reactions.

PCR product	CFU/ml	Fold Increase
B1-tet-B2	7,500	
B1.6-tet-B2	12,000	1.6 x
B1-tet-B2.10	20,900	2.8 x
B1.6-tet-B2.10	30,100	4.0 x

These results demonstrate that attB PCR products containing sequences that perfectly match the proposed consensus sequence for integrase core binding sites can produce Entry clones with four-fold higher efficiency than standard Gateway attB1 and attB2 PCR products.

The entry clones produced above were then transferred to pDEST20 (Figure 40A) via LR reactions (300 ng (64 fmoles) pDEST20 mixed with 50 ng (77 fmoles) of the respective pENTR201-TET Entry clone in 20 µl volume; incubated for 1 hr incubation at 25°C). The efficiencies of cloning for these reactions are compared in Table 6.

Table 6. Cloning Efficiency of LR Reactions.

pENTR201-TET x pDEST20	CFU/ml	Fold Increase
L1-tet-L2	5,800	
L1.6-tet-L2	8,000	1.4
L1-tet-L2.10	10,000	1.7
L1.6-tet-L2.10	9,300	1.6

These results demonstrate that the mutations introduced into attB1.6 and attB2.10 that transfer with the gene into entry clones slightly increase the efficiency of LR reactions. Thus, the present invention encompasses not only mutations in *attB* sites that increase recombination efficiency, but also to the corresponding mutations that result in the *attL* sites created by the BP reaction.

To examine the increased cloning efficiency of the attB1.6-TET-attB2.10 PCR product over a range of PCR product amounts, experiments analogous to those described above were performed in which the amount of attB PCR product was titrated into the reaction mixture. The results are shown in Table 7.

Table 7. Titration of attB PCR products.

Amount of attB PCR product (ng)	PCR product	CFU/ml	Fold Increase
20	attB1-TET-attB2	3,500	6.1
	attB1.6-TET-attB2.10	21,500	
50	attB1-TET-attB2	9,800	5.0
	attB1.6-TET-attB2.10	49,000	
100	attB1-TET-attB2	18,800	2.8
	attB1.6-TET-attB2.10	53,000	
200	attB1-TET-attB2	19,000	2.5
	attB1.6-TET-attB2.10	48,000	

These results demonstrate that as much as a six-fold increase in cloning efficiency is achieved with the attB1.6-TET-attB2.10 PCR product as compared to the standard attB1-TET-attB2 PCR product at the 20 ng amount.

Example 24: Determination of attB Sequence Requirements for Optimum Recombination Efficiency

To examine the sequence requirements for attB and to determine which attB sites would clone with the highest efficiency from populations of degenerate attB sites, a series of experiments was performed. Degenerate PCR primers were designed which contained five bases of degeneracy in the B-arm of the attB site. These degenerate sequences would thus transfer with the gene into Entry clone in BP reactions and subsequently be transferred with the gene into expression clones in LR reactions. The populations of degenerate attB and attL sites could thus be cycled from attB to attL back and forth for any number of cycles. By altering the reaction conditions at each transfer step (for example by decreasing the reaction time and/or decreasing the concentration of DNA) the reaction can be made increasingly more stringent at each cycle and thus enrich for populations of attB and attL sites that react more efficiently.

The following degenerate PCR primers were used to amplify a 500 bp fragment from pUC18 which contained the lacZ alpha fragment (only the attB portion of each primer is shown):

```
attB1      GGGG ACAAGTTTGTACAAA AAAGC AGGCT
attB1n16-20 GGGG ACAAGTTTGTACAAA nnnnn AGGCT
attB1n21-25 GGGG ACAAGTTTGTACAAA AAAGC nnnnn

attB2      GGGG ACCACTTTGTACAAG AAAGC TGGGT
attB2n16-20 GGGG ACCACTTTGTACAAG nnnnn TGGGT
attB2n21-25 GGGG ACCACTTTGTACAAG AAAGC nnnnn
```

The starting population size of degenerate att sites is 4^5 or 1024 molecules. Four different populations were transferred through two BP reactions and two LR reactions. Following transformation of each reaction, the population of transformants was amplified by growth in liquid media containing the appropriate selection antibiotic. DNA was prepared from the population of clones by alkaline

-161-

lysis miniprep and used in the next reaction. The results of the BP and LR cloning reactions are shown below.

BP-1, overnight reactions

	cfu/ml	percent of control
attB1-LacZa-attB2	78,500	100 %
attB1n16-20-LacZa-attB2	1,140	1.5 %
attB1n21-25-LacZa-attB2	11,100	14 %
attB1-LacZa-attB2n16-20	710	0.9 %
attB1-LacZa-attB2n21-25	16,600	21 %

LR-1, pENTR201-LacZa x pDEST20/*EcoRI*, 1hr reactions

	cfu/ml	percent of control
attL1-LacZa-attL2	20,000	100 %
attL1n16-20-LacZa-attL2	2,125	11 %
attL1n21-25-LacZa-attL2	2,920	15 %
attL1-LacZa-attL2n16-20	3,190	16 %
attL1-LacZa-attL2n21-25	1,405	7 %

BP-2, pEXP20-LacZa/*ScaI* x pDONR 201, 1hr reactions

	cfu/ml	percent of control
attB1-LacZa-attB2	48,600	100 %
attB1n16-20-LacZa-attB2	22,800	47 %
attB1n21-25-LacZa-attB2	31,500	65 %
attB1-LacZa-attB2n16-20	42,400	87 %
attB1-LacZa-attB2n21-25	34,500	71 %

LR-2, pENTR201-LacZa x pDEST6/*NcoI*, 1hr reactions

	cfu/ml	percent of control
attL1-LacZa-attL2	23,000	100 %
attL1n16-20-LacZa-attL2	49,000	213 %
attL1n21-25-LacZa-attL2	18,000	80 %
attL1-LacZa-attL2n16-20	37,000	160 %
attL1-LacZa-attL2n21-25	57,000	250 %

These results demonstrate that at each successive transfer, the cloning efficiency of the entire population of att sites increases, and that there is a great deal of flexibility in the definition of an attB site. Specific clones may be isolated from the above reactions, tested individually for recombination efficiency, and

sequenced. Such new specificities may then be compared to known examples to guide the design of new sequences with new recombination specificities. In addition, based on the enrichment and screening protocols described herein, one of ordinary skill can easily identify and use sequences in other recombination sites, *e.g.*, other *att* sites, *lox*, FRT, etc., that result in increased specificity in the recombination reactions using nucleic acid molecules containing such sequences.

Example 25: Design of att Site PCR Adapter-Primers

Additional studies were performed to design gene-specific primers with 12bp of attB1 and attB2 at their 5'-ends. The optimal primer design for *att*-containing primers is the same as for any PCR primers: the gene-specific portion of the primers should ideally have a T_m of $> 50^\circ\text{C}$ at 50 mM salt (calculation of T_m is based on the formula $59.9 + 41(\%GC) - 675/n$).

Primers:

12bp attB1: AA AAA GCA GGC TNN - forward gene-specific primer

12bp attB2: A GAA AGC TGG GTN - reverse gene-specific primer

attB1 adapter primer: GGGGACAAGTTTGTACAAAAAAGCAGGCT

attB2 adapter primer: GGGGACCACTTTGTACAAGAAAGCTGGGT

Protocol:

(1) Mix 200 ng of cDNA library or 1 ng of plasmid clone DNA (alternatively, genomic DNA or RNA could be used) with 10 pmoles of gene specific primers in a 50 μl PCR reaction, using one or more polypeptides having DNA polymerase activity such as those described herein. (The addition of greater than 10 pmoles of gene-specific primers can decrease the yield of attB PCR product. In addition, if RNA is used, a standard reverse transcriptase-PCR (RT-

PCR) protocol should be followed; *see, e.g.,* Gerard, G.F., *et al.*, *FOCUS* 11:60 (1989); Myers, T.W., and Gelfand, D.H., *Biochem.* 30:7661 (1991); Freeman, W.N., *et al.*, *BioTechniques* 20:782 (1996); and U.S. Application No. 09/064,057, filed April 22, 1998, the disclosures of all of which are incorporated herein by reference.)

1st PCR profile:

(a) 95°C for 3 minutes

(b) 10 cycles of:

(i) 94°C for 15 seconds

(ii) 50°C* for 30 seconds

(iii) 68°C for 1 minute/kb of target amplicon

(c) 68°C for 5 minutes

(d) 10°C hold

*The optimal annealing temperature is determined by the calculated T_m of the gene-specific part of the primer.

(2) Transfer 10 µl to a 40 µl PCR reaction mix containing 35 pmoles each of the attB1 and attB2 adapter primers.

2nd PCR profile:

(a) 95°C for 1 minute

(b) 5 cycles of:

(i) 94°C for 15 seconds

(ii) 45°C* for 30 seconds

(iii) 68°C for 1 minute/kb of target amplicon

(c) 15-20 cycles** of:

(i) 94°C for 15 seconds

(ii) 55°C* for 30 seconds

(iii) 68°C for 1 minute/kb of target amplicon

(d) 68°C for 5 minutes

(e) 10°C hold

*The optimal annealing temperature is determined by the calculated T_m of the gene-specific part of the primer.

**15 cycles is sufficient for low complexity targets.

Notes:

1. It is useful to perform a no-adaptor primer control to assess the yield of attB PCR product produced.
2. Linearized template usually results in slightly greater yield of PCR product.

Example 26: One-Tube Recombinational Cloning Using the GATEWAY™ Cloning System

To provide for easier and more rapid cloning using the GATEWAY™ cloning system, we have designed a protocol whereby the BP and LR reactions may be performed in a single tube (a "one-tube" protocol). The following is an example of such a one-tube protocol; in this example, an aliquot of the BP reaction is taken before adding the LR components, but the BP and LR reactions may be performed in a one-tube protocol without first taking the BP aliquot:

<u>Reaction Component</u>	<u>Volume</u>
attB DNA (100-200 ng/25 µl reaction)	1-12.5 µl
attP DNA (pDONR201) 150 ng/µl	2.5 µl
5X BP Reaction Buffer	5.0 µl
Tris-EDTA	(to 20 µl)
<u>BP Clonase</u>	<u>5.0 µl</u>
Total vol.	25 µl

After the above components were mixed in a single tube, the reaction mixtures were incubated for 4 hours at 25°C. A 5 µl aliquot of reaction mixture was removed, and 0.5 µl of 10X stop solution was added to this reaction mixture and incubated for 10 minutes at 37°C. Competent cells were then transformed with 1-2 µl of the BP reaction per 100 µl of cells; this transformation yielded colonies of Entry Clones for isolation of individual Entry Clones and for quantitation of the BP Reaction efficiency.

To the remaining 20 µl of BP reaction mixture, the following components of the LR reaction were added:

<u>Reaction Component</u>	<u>Final Concentration</u>	<u>Volume Added</u>
NaCl	0.75 M	1 µl
Destination Vector	150 ng/ul	3 µl
<u>LR Clonase</u>		<u>6 µl</u>
Total vol.		30 µl

After the above components were mixed in a single tube, the reaction mixtures were incubated for 2 hours at 25°C. 3 µl of 10X stop solution was added, and the mixture was incubated for 10 minutes at 37°C. Competent cells were then transformed with 1-2 µl of the reaction mixture per 100 µl of cells

Notes:

1. If desired, the Destination Vector can be added to the initial BP reaction.
2. The reactions can be scaled down by 2x, if desired.
3. Shorter incubation times for the BP and/or LR reactions can be used (scaled to the desired cloning efficiencies of the reaction), but a lower number of colonies will typically result.
4. To increase the number of colonies obtained by several fold, incubate the BP reaction for 6-20 hours and increase the LR reaction to 3 hours. Electroporation also works well with 1-2 ul of the PK-treated reaction mixture.

5. PCR products greater than about 5 kb may show significantly lower cloning efficiency in the BP reaction. In this case, we recommend using a one-tube reaction with longer incubation times (*e.g.*, 6-18 hours) for both the BP and LR steps.

Example 27: Relaxation of Destination Vectors During the LR Reaction

To further optimize the LR Reaction, the composition of the LR Reaction buffer was modified from that described above and this modified buffer was used in a protocol to examine the impact of enzymatic relaxation of Destination Vectors during the LR Reaction.

LR Reactions were set up as usual (*see, e.g.*, Example 6), except that 5X BP Reaction Buffer (*see* Example 5) was used for the LR Reaction. To accomplish Destination Vector relaxation during the LR Reaction, Topoisomerase I (Life Technologies, Inc., Rockville, MD; Catalogue No. 38042-016) was added to the reaction mixture at a final concentration of ~15U per μg of total DNA in the reaction (for example, for reaction mixtures with a total of 400ng DNA in the 20 μl LR Reaction, ~6units of Topoisomerase I was added). Reaction mixtures were set up as follows:

<u>Reaction Component</u>	<u>Volume</u>
ddH ₂ O	6.5 μl
4X BP Reaction Buffer	5 μl
100ng single chain/linear pENTR CAT, 50 ng/ μl	2 μl
300ng single chain/linear pDEST6, 150ng/ μl	2 μl
Topoisomerase I, 15 U/ml	0.5 μl
LR Clonase	4 μl

Reaction mixtures were incubated at 25°C for 1 hour, and 2 μl of 2 $\mu\text{g}/\mu\text{l}$ Proteinase K was then added and mixtures incubated for 10 minutes at 37°C to stop the LR Reaction. Competent cells were then transformed as described in the preceding examples. The results of these studies demonstrated that relaxation of

substrates in the LR reaction using Topoisomerase I resulted in a 2- to 10-fold increase in colony output compared to those LR reactions performed without including Topoisomerase I.

5 Having now fully described the present invention in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious to one of ordinary skill in the art that the same can be performed by modifying or changing the invention within a wide and equivalent range of conditions, formulations and other parameters without affecting the scope of the invention or
10 any specific embodiment thereof, and that such modifications or changes are intended to be encompassed within the scope of the appended claims.

 All publications, patents and patent applications mentioned in this specification are indicative of the level of skill of those skilled in the art to which this invention pertains, and are herein incorporated by reference to the same extent
15 as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference.

167.1

Applicant's or agent's file reference number	0942.558PC03	International application No. ^{tl}	PCT/US 00/05432
---	--------------	---	-----------------

**INDICATIONS RELATING TO DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL**
(PCT Rule 13bis)

REC'D 17 APR 2000

WIPO

PCT

A. The indications made below relate to the microorganism referred to in the description on page 52, line 31.

B. IDENTIFICATION OF DEPOSITFurther deposits are identified on an additional sheet ☒

Name of depositary institution
Agricultural Research Culture Collection (NRRL)
International Depository Authority

Address of depositary institution (including postal code and country)

1815 N. University Street
Peoria, Illinois 61604
United States of America

Date of deposit
February 27, 1999

Accession Number
NRRL B-30099

C. ADDITIONAL INDICATIONS (leave blank if not applicable)This information is continued on an additional sheet ☐

Escherichia coli DB3.1(pAHPKan) or Escherichia coli DB3.1(pAttPKan)

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)**E. SEPARATE FURNISHING OF INDICATIONS** (leave blank if not applicable)

The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")

For receiving Office use only

☒ This sheet was received with the international application

Authorized officer

Signature: [Signature]
IT Specialist, WIPO Technical
Department - 3005-2200 (FA)

For International Bureau use only

☐ This sheet was received by the International Bureau on:

Authorized officer

167.2

Applicant's or agent's file reference number	0942.468PC03	International application No. tl	PCT/US 00/05432
--	--------------	----------------------------------	-----------------

**INDICATIONS RELATING TO DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL**
(PCT Rule 13bis)

REC'D 17 APR 2000

WIPO PGT

A. The indications made below relate to the microorganism referred to in the description on page 55, line 16.

B. IDENTIFICATION OF DEPOSITFurther deposits are identified on an additional sheet ☒

Name of depositary institution
Agricultural Research Culture Collection (NRRL)
International Depository Authority

Address of depositary institution (including postal code and country)

1815 N. University Street
Peoria, Illinois 61604
United States of America

Date of deposit
February 27, 1999

Accession Number
NRRL B-30100

C. ADDITIONAL INDICATIONS (leave blank if not applicable)This information is continued on an additional sheet ☐

Escherichia coli DB3.1(pENTR-1A)

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)**E. SEPARATE FURNISHING OF INDICATIONS** (leave blank if not applicable)

The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")

For receiving Office use only

☒ This sheet was received with the international application

Authorized officer

Barbara Fridis

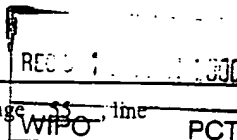
For International Bureau use only

☐ This sheet was received by the International Bureau on:

Authorized officer

Applicant's or agent's file reference number	0942.468PC03	International application No. 1b- PCT/US 00/05432
---	--------------	--

**INDICATIONS RELATING TO DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL**
(PCT Rule 13bis)



A. The indications made below relate to the microorganism referred to in the description on page 16, line 16.	WIPO PCT
---	----------

B. IDENTIFICATION OF DEPOSIT		Further deposits are identified on an additional sheet <input checked="" type="checkbox"/>
Name of depositary institution Agricultural Research Culture Collection (NRRL) International Depository Authority		
Address of depositary institution (including postal code and country) 1815 N. University Street Peoria, Illinois 61604 United States of America		
Date of deposit February 27, 1999	Accession Number NRRL B-30101	

C. ADDITIONAL INDICATIONS (leave blank if not applicable)	This information is continued on an additional sheet <input type="checkbox"/>
Escherichia coli DB3.1(pENTR-2B)	

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)

E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)
The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")

For receiving Office use only	For International Bureau use only
<input checked="" type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer Daria Fries PCT Section, WIPO Tel: +41 22 338 9230 (Ext. 311)	Authorized officer

167.4

Applicant's or agent's file reference number	0942.468PC03	International Application No. PCT/US 0/05432
--	--------------	---

**INDICATIONS RELATING TO DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL**
(PCT Rule 13bis)

REC'D 17 APR 2000

WIPO PCT

A. The indications made below relate to the microorganism referred to in the description on page 55, line 16.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☒

Name of depositary institution
Agricultural Research Culture Collection (NRRL)
International Depositary Authority

Address of depositary institution (including postal code and country)

1815 N. University Street
Peoria, Illinois 61604
United States of America

Date of deposit
February 27, 1999

Accession Number
NRRL B-30102

C. ADDITIONAL INDICATIONS (leave blank if not applicable)

This information is continued on an additional sheet ☐

Escherichia coli DB3.1(pENTR-3C)

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)

E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)

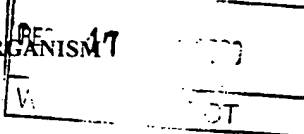
The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")

For receiving Office use only	For International Bureau use only
<input checked="" type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer <i>[Signature]</i> 12/20/99 125-3220 (F)	Authorized officer

167.5

Applicant's or agent's file reference number	0942.468PC03	International application No. tb. PCT/US 00/05432
---	--------------	---

**INDICATIONS RELATING TO DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL**
(PCT Rule 13bis)



A. The indications made below relate to the microorganism referred to in the description on page <u>8</u> .	REC-17 APR 2000 WIPO PCT
---	-----------------------------

B. IDENTIFICATION OF DEPOSIT		Further deposits are identified on an additional sheet <input checked="" type="checkbox"/>
Name of depositary institution Agricultural Research Culture Collection (NRRL) International Depositary Authority		
Address of depositary institution (including postal code and country) 1815 N. University Street Peoria, Illinois 61604 United States of America		
Date of deposit February 27, 1999	Accession Number NRRL B-30103	

C. ADDITIONAL INDICATIONS (leave blank if not applicable)	This information is continued on an additional sheet <input type="checkbox"/>
Escherichia coli DB3.1(pEZC15101)	

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)

E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)
The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")

For receiving Office use only	For International Bureau use only
<input checked="" type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer TEODORA ANTONIU 15-03-2000 (E)	Authorized officer

167.6

Applicant's or agent's file reference number	0942.468PC03	International application No. 1.	PCT/US 00/05432
---	--------------	----------------------------------	-----------------

**INDICATIONS RELATING TO DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL**
(PCT Rule 13bis)

REC'D 17

VPO

A. The indications made below relate to the microorganism referred to in the description on page 54, line 9.

B. IDENTIFICATION OF DEPOSITFurther deposits are identified on an additional sheet ☒

Name of depositary institution
Agricultural Research Culture Collection (NRRL)
International Depository Authority

Address of depositary institution (including postal code and country)

1815 N. University Street
Peoria, Illinois 61604
United States of America

Date of deposit
February 27, 1999

Accession Number
NRRL B-30104

C. ADDITIONAL INDICATIONS (leave blank if not applicable)This information is continued on an additional sheet ☐

Escherichia coli DB3.1(pEZC15102)

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)**E. SEPARATE FURNISHING OF INDICATIONS** (leave blank if not applicable)

The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")

For receiving Office use only

For International Bureau use only

☒ This sheet was received with the international application

☐ This sheet was received by the International Bureau on:

Authorized officer

Barbara F. H. H. H.

Authorized officer

167.7

Applicant's or agent's file reference number	0942.468PC03	International application No. PCT/US	00/05432
---	--------------	---	-----------------

**INDICATIONS RELATING TO DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL**
(PCT Rule 13bis)

RECEIVED 17 APR 1999

V. T

A. The indications made below relate to the microorganism referred to in the description on page 54, line 9.

B. IDENTIFICATION OF DEPOSITFurther deposits are identified on an additional sheet ☒

Name of depositary institution
Agricultural Research Culture Collection (NRRL)
International Depository Authority

Address of depositary institution (including postal code and country)

1815 N. University Street
Peoria, Illinois 61604
United States of America

Date of deposit
February 27, 1999

Accession Number
NRRL B-30105

C. ADDITIONAL INDICATIONS (leave blank if not applicable)This information is continued on an additional sheet ☐

Escherichia coli DB3.1(pEZC15103)

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)**E. SEPARATE FURNISHING OF INDICATIONS** (leave blank if not applicable)

The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")

For receiving Office use only

<input checked="" type="checkbox"/> This sheet was received with the international application
Authorized officer <i>[Signature]</i>

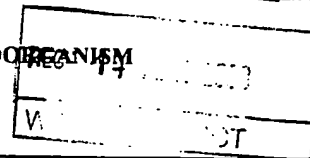
For International Bureau use only

<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer

167.8

Applicant's or agent's file reference number	0942.408PC03	International application No. tl PCT/US 00/05432
---	--------------	--

**INDICATIONS RELATING TO DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL**
(PCT Rule 13bis)



A. The indications made below relate to the microorganism referred to in the description on page <u>51</u> , line <u>20-21</u> .	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input checked="" type="checkbox"/>	
Name of depositary institution Agricultural Research Culture Collection (NRRL) International Depository Authority	
Address of depositary institution (including postal code and country) 1815 N. University Street Peoria, Illinois 61604 United States of America	
Date of deposit February 27, 1999	Accession Number NRRL B-30108
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
Escherichia coli DB10B(pCMVSPORT6)	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")	

For receiving Office use only	For International Bureau use only
<input checked="" type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer Barbara F. [illegible] <i>BF</i>	Authorized officer

WHAT IS CLAIMED IS:

1. An isolated nucleic acid molecule comprising a nucleotide sequence selected from the group of nucleotide sequences consisting of an attB1 nucleotide sequence as set forth in Figure 9, an attB2 nucleotide sequence as set forth in Figure 9, an attP1 nucleotide sequence as set forth in Figure 9, an attP2 nucleotide sequence as set forth in Figure 9, an attL1 nucleotide sequence as set forth in Figure 9, an attL2 nucleotide sequence as set forth in Figure 9, an attR1 nucleotide sequence as set forth in Figure 9, an attR2 nucleotide sequence as set forth in Figure 9, a polynucleotide complementary thereto, and a mutant, fragment, or derivative thereof.

2. An isolated nucleic acid molecule comprising an attB1 nucleotide sequence as set forth in Figure 9, a polynucleotide complementary thereto, or a mutant, fragment, variant or derivative thereof.

3. An isolated nucleic acid molecule comprising an attB2 nucleotide sequence as set forth in Figure 9, a polynucleotide complementary thereto, or a mutant, fragment, variant or derivative thereof.

4. An isolated nucleic acid molecule comprising an attP1 nucleotide sequence as set forth in Figure 9, a polynucleotide complementary thereto, or a mutant, fragment, variant or derivative thereof.

5. An isolated nucleic acid molecule comprising an attP2 nucleotide sequence as set forth in Figure 9, a polynucleotide complementary thereto, or a mutant, fragment, variant or derivative thereof.

6. An isolated nucleic acid molecule comprising an attL1 nucleotide sequence as set forth in Figure 9, a polynucleotide complementary thereto, or a mutant, fragment, variant or derivative thereof.

7. An isolated nucleic acid molecule comprising an attL2 nucleotide sequence as set forth in Figure 9, a polynucleotide complementary thereto, or a mutant, fragment, variant or derivative thereof.

5 8. An isolated nucleic acid molecule comprising an attR1 nucleotide sequence as set forth in Figure 9, a polynucleotide complementary thereto, or a mutant, fragment, variant or derivative thereof.

10 9. An isolated nucleic acid molecule comprising an attR2 nucleotide sequence as set forth in Figure 9, a polynucleotide complementary thereto, or a mutant, fragment, variant or derivative thereof.

15 10. The isolated nucleic acid molecule of claim 1, further comprising one or more functional or structural nucleotide sequences selected from the group consisting of one or more multiple cloning sites, one or more localization signals, one or more transcription termination sites, one or more transcriptional regulatory sequences, one or more translational signals, one or more origins of replication, one or more fusion partner peptide-encoding nucleic acid molecules, one or more protease cleavage sites, and one or more 5' polynucleotide extensions.

20 11. The nucleic acid molecule of claim 10, wherein said transcriptional regulatory sequence is a promoter, an enhancer, or a repressor.

25 12. The nucleic acid molecule of claim 10, wherein said fusion partner peptide-encoding nucleic acid molecule encodes glutathione S-transferase (GST), hexahistidine (His₆), or thioredoxin (Trx).

30 13. The nucleic acid molecule of claim 10, wherein said 5' polynucleotide extension consists of from one to five nucleotide bases.

14. The nucleic acid molecule of claim 13, wherein said 5' polynucleotide extension consists of four or five guanine nucleotide bases.

15. A primer nucleic acid molecule suitable for amplifying a target nucleotide sequence, comprising the isolated nucleic acid molecule of claim 1 or a portion thereof linked to a target-specific nucleotide sequence useful in amplifying said target nucleotide sequence.

16. The primer nucleic acid molecule of claim 15, wherein said primer comprises an attB1 nucleotide sequence having the sequence shown in Figure 9 or a portion thereof, or a polynucleotide complementary to the sequence shown in Figure 9 or a portion thereof.

17. The primer nucleic acid molecule of claim 15, wherein said primer comprises an attB2 nucleotide sequence having the sequence shown in Figure 9 or a portion thereof, or a polynucleotide complementary to the sequence shown in Figure 9 or a portion thereof.

18. The primer nucleic acid molecule of claim 15, further comprising a 5' terminal extension of four or five guanine bases.

19. A vector comprising the isolated nucleic acid molecule of claim 1.

20. The vector of claim 19, wherein said vector is an Expression Vector.

21. A host cell comprising the isolated nucleic acid molecule of claim 1 or the vector of claim 19.

22. A method of synthesizing or amplifying one or more nucleic acid molecules comprising:

- (a) mixing one or more nucleic acid templates with at least one polypeptide having polymerase or reverse transcriptase activity and at least a first primer comprising a template-specific sequence that is complementary to or capable of hybridizing to said

templates and at least a second primer comprising all or a portion of a recombination site wherein said at least a portion of said second primer is homologous to or complementary to at least a portion of said first primer; and

- 5 (b) incubating said mixture under conditions sufficient to synthesize or amplify one or more nucleic acid molecules complementary to all or a portion of said templates and comprising one or more recombination sites or portions thereof at one or both termini of said molecules.

10 23. A method of synthesizing or amplifying one or more nucleic acid molecules comprising:

- 15 (a) mixing one or more nucleic acid templates with at least one polypeptide having polymerase or reverse transcriptase activity and at least a first primer comprising a template-specific sequence that is complementary to or capable of hybridizing to said templates and at least a portion of a recombination site, and at least a second primer comprising all or a portion of a recombination site wherein said at least a portion of said recombination site on said second primer is complementary to or homologous to at least a portion of said recombination site on said first primer; and

- 20 (b) incubating said mixture under conditions sufficient to synthesize or amplify one or more nucleic acid molecules complementary to all or a portion of said templates and comprising one or more recombination sites or portions thereof at one or both termini of said molecules.

25 24. A method of amplifying or synthesizing one or more nucleic acid molecules comprising:

- 30 (a) mixing one or more nucleic acid templates with at least one polypeptide having polymerase or reverse transcriptase activity

and one or more first primers comprising at least a portion of a recombination site and a template-specific sequence that is complementary to or capable of hybridizing to said template;

(b) incubating said mixture under conditions sufficient to synthesize or amplify one or more first nucleic acid molecules complementary to all or a portion of said templates wherein said molecules comprise at least a portion of a recombination site at one or both termini of said molecules;

(c) mixing said molecules with one or more second primers comprising one or more recombination sites, wherein said recombination sites of said second primers are homologous to or complementary to at least a portion of said recombination sites on said first nucleic acid molecules; and

(d) incubating said mixture under conditions sufficient to synthesize or amplify one or more second nucleic acid molecules complementary to all or a portion of said first nucleic acid molecules and which comprise one or more recombination sites at one or both termini of said molecules.

25. A polypeptide encoded by the isolated nucleic acid molecule of any one of claims 1-10.

26. An isolated nucleic acid molecule comprising one or more *att* recombination sites comprising at least one mutation in its core region that increases the specificity of interaction between said recombination site and a second *att* recombination site.

27. The isolated nucleic acid molecule of claim 26, wherein said mutation is at least one substitution mutation of at least one nucleotide in the seven basepair overlap region of said core region of said recombination site.

28. The isolated nucleic acid molecule of claim 26, wherein said nucleic acid molecule comprises the sequence NNNATAC, wherein "N" refers to any nucleotide with the proviso that if one of the first three nucleotides in the consensus sequence is a T/U, then at least one of the other two of the first three nucleotides is not a T/U.

29. An isolated nucleic acid molecule comprising one or more mutated *att* recombination sites comprising at least one mutation in its core region that enhances the efficiency of recombination between a first nucleic acid molecule comprising said mutated *att* recombination site and a second nucleic acid molecule comprising a second recombination site that interacts with said mutated *att* recombination site.

30. The isolated nucleic acid molecule of claim 29, wherein said mutated *att* recombination site is a mutated *attL* site comprising a core region having the nucleotide sequence caactntntnnnannaagttg, wherein "n" represents any nucleotide.

31. The isolated nucleic acid molecule of claim 30, wherein said mutated *attL* recombination site comprises a core region having a nucleotide sequence selected from agcctgctttattataactaagttggcatta (*attL5*) and agcctgctttttatattaagttggcatta (*attL6*).

32. The isolated nucleic acid molecule of claim 29, wherein said mutated *att* recombination site comprises a core region having a nucleotide sequence selected from the group consisting of ggggacaactttgtacaaaaagttggct (*attB1.6*), ggggacaactttgtacaagaagctgggt (*attB2.2*), and ggggacaactttgtacaagaagttgggt (*attB2.10*).

33. A vector selected from the group consisting of pENTR1A, pENTR2B, pENTR3C, pENTR4, pENTR5, pENTR6, pENTR7, pENTR8, pENTR9, pENTR10, pENTR11, pDEST1, pDEST2, pDEST3, pDEST4,

pDEST5, pDEST6, pDEST7, pDEST8, pDEST9, pDEST10, pDEST11, pDEST12.2 (also known as pDEST12), pDEST13, pDEST14, pDEST15, pDEST16, pDEST17, pDEST18, pDEST19, pDEST20, pDEST21, pDEST22, pDEST23, pDEST24, pDEST25, pDEST26, pDEST27, pDEST28, pDEST29, pDEST30, pDEST31, pDEST32, pDEST33, pDEST34, pDONR201 (also known as pENTR21 attP vector or pAttPkan Donor Vector), pDONR202, pDONR203 (also known as pEZ15812), pDONR204, pDONR205, pDONR206 (also known as pENTR22 attP vector or pAttPgen Donor Vector), pDONR207, pMAB58, pMAB62, pMAB85 and pMAB86.

34. A host cell comprising the vector of claim 33.

35. A polypeptide encoded by the vector of claim 33.

36. A kit for use in synthesizing a nucleic acid molecule, said kit comprising the isolated nucleic acid molecule of any one of claims 1-10, 26 and 29.

37. A kit for use in synthesizing a nucleic acid molecule, said kit comprising the primer of claim 15 or claim 18.

38. A kit for use in cloning a nucleic acid molecule, said kit comprising the vector of claim 19 or claim 33.

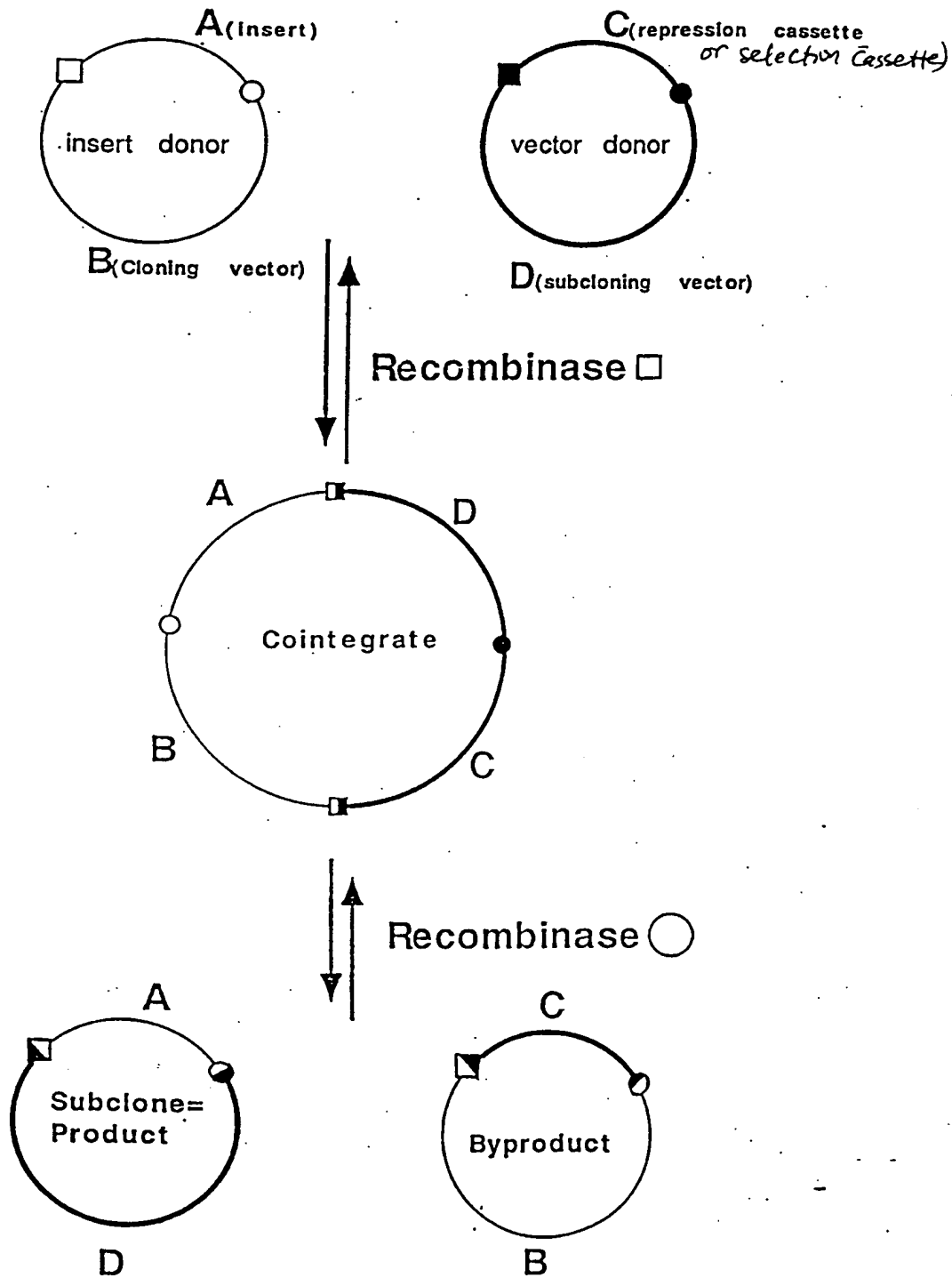


Figure 1

2/240

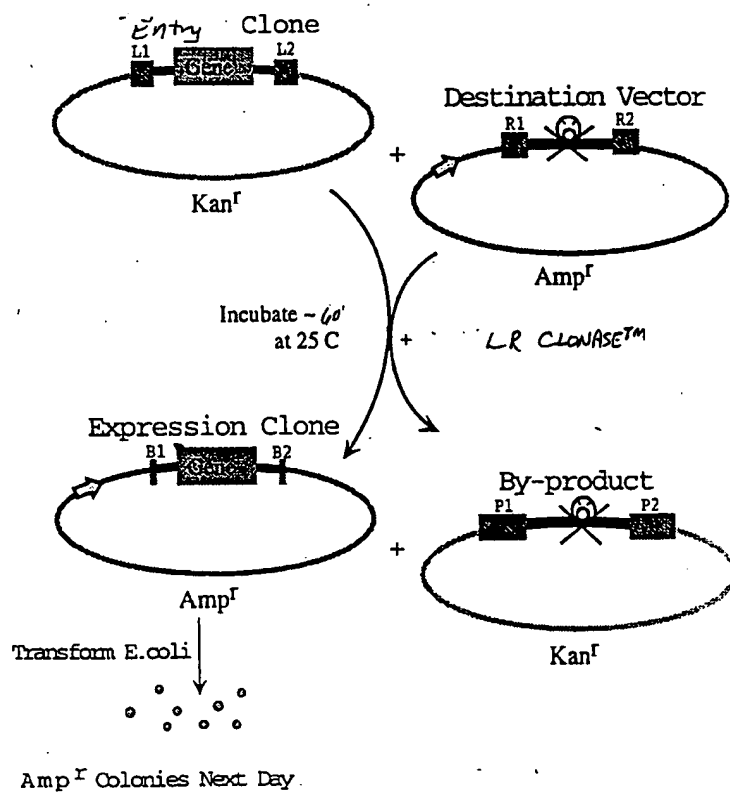


FIGURE 2

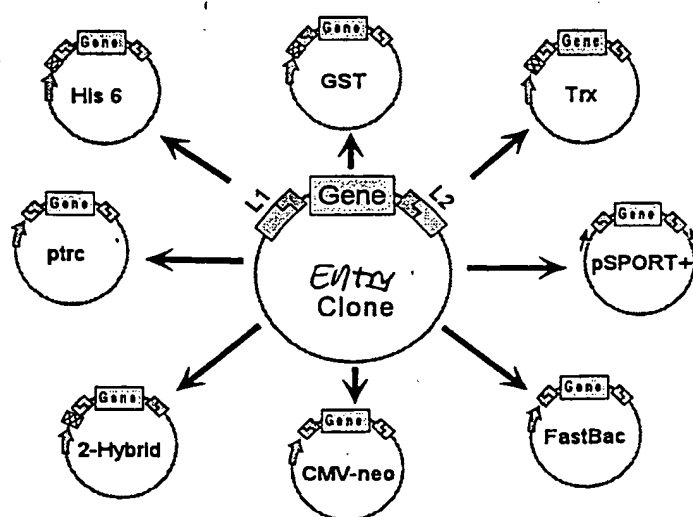


FIGURE 3

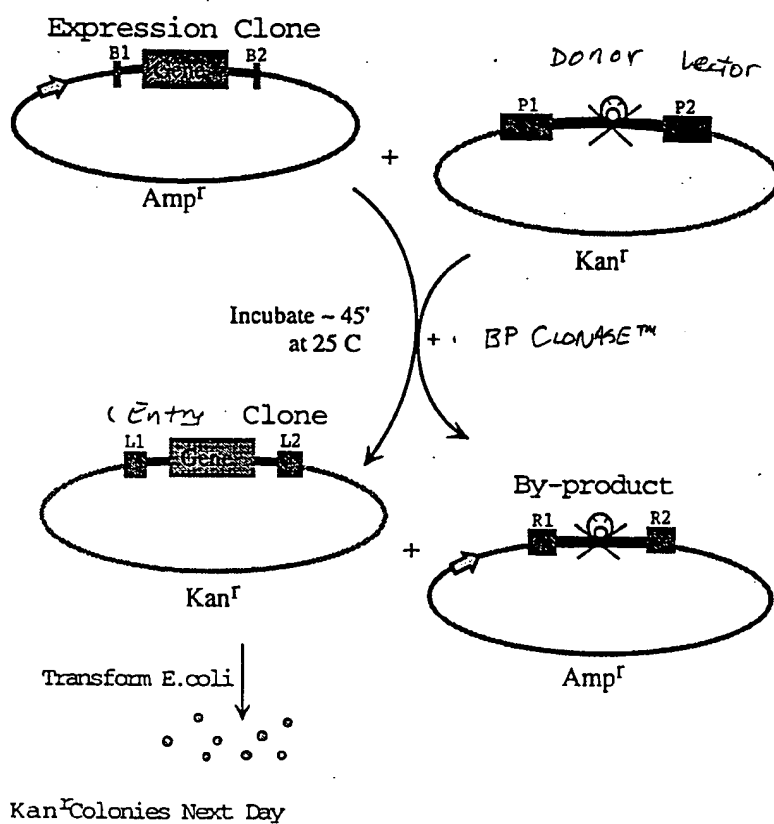


FIGURE 4

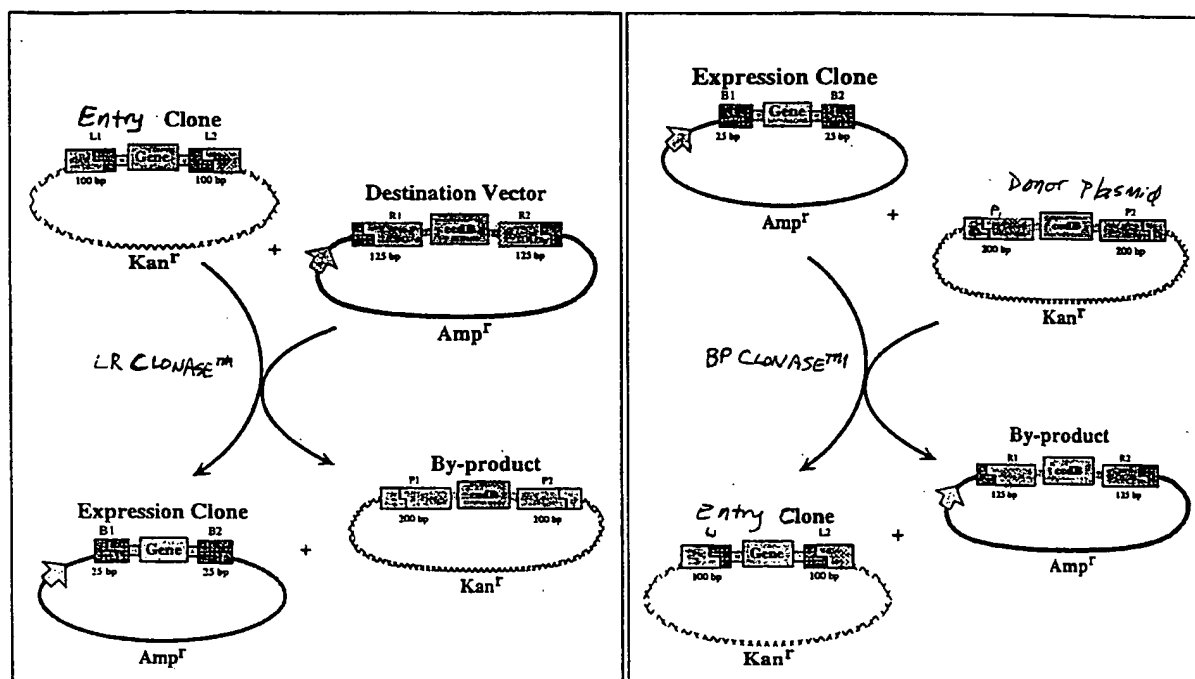


FIGURE 5

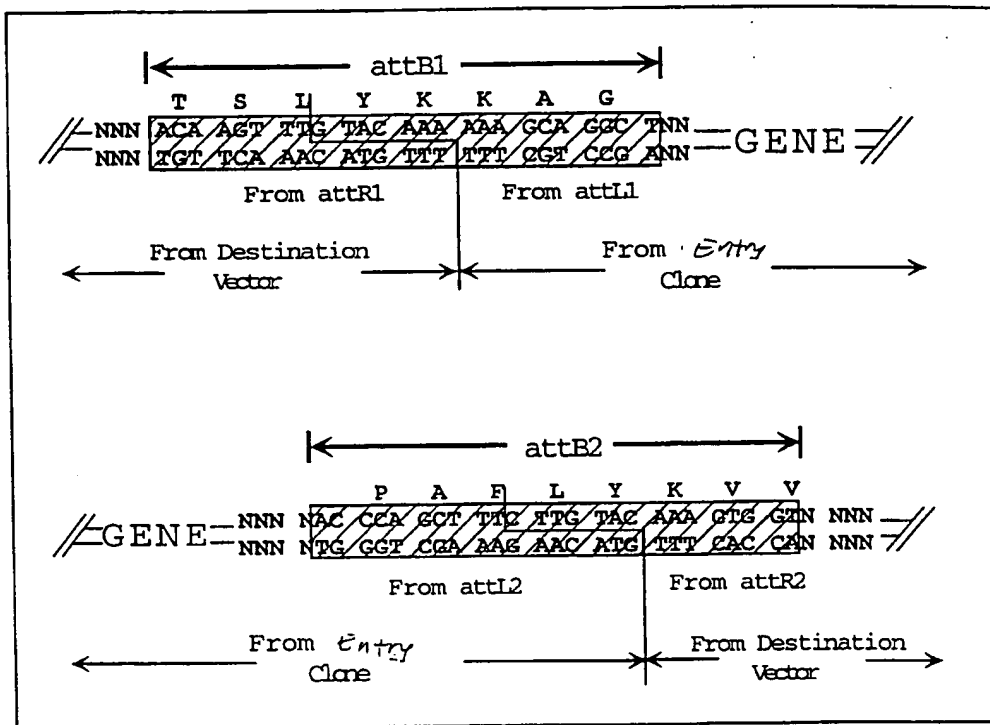


FIGURE 6

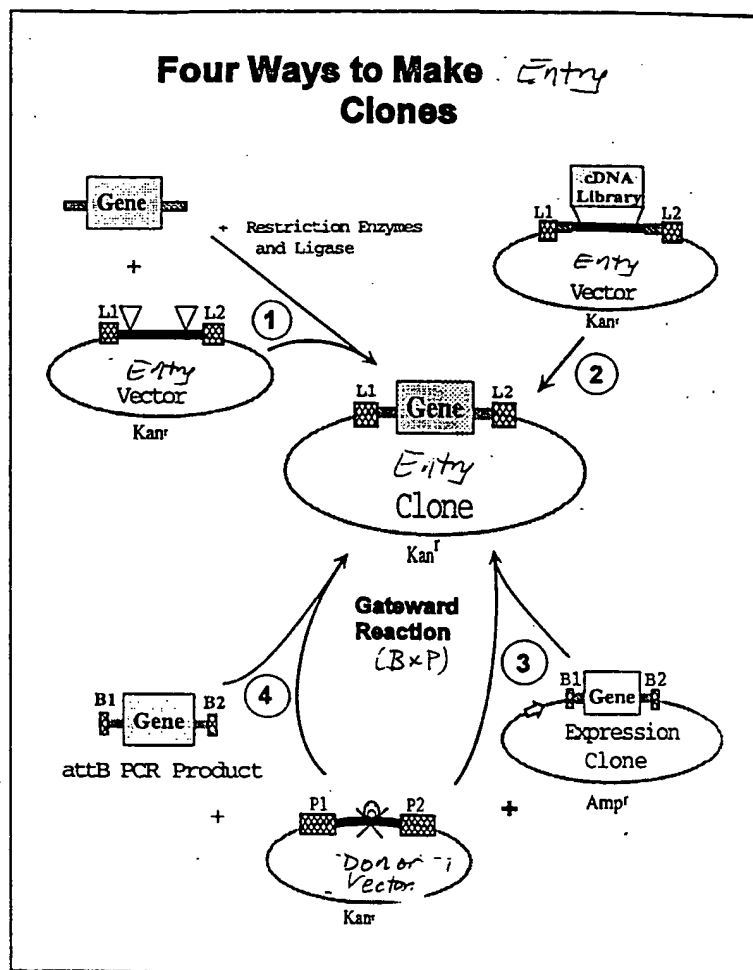


FIGURE 7

8/240

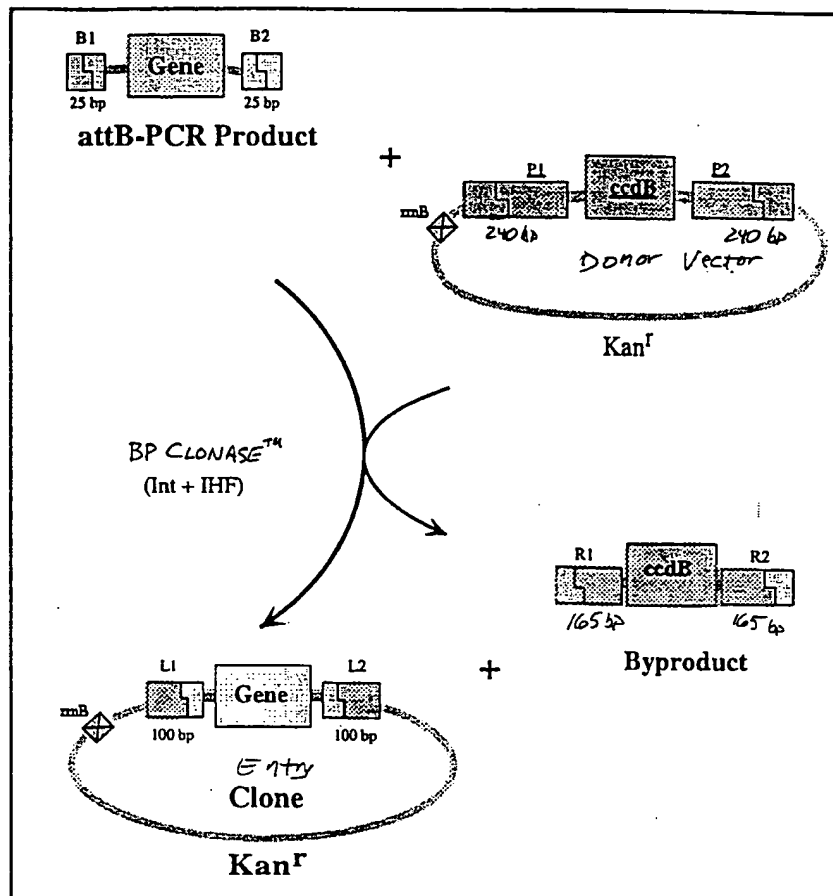


FIGURE 8

Recombination Site Nucleotide Sequences

attB1: 5'-ACAAGTTTGTACAAAAAAGCAGGCT-3'

attB2: 5'-ACCCAGCTTTCTTGTACAAAGTGGT-3'

attP1: 5'-TACAGGTCCTAATACCATCTAAGTAGTTGATTCATAGTGACTGGATATG-TTGTGTTTTACAGTATTATGTAGTCTGTTTTTATGCAAAATCTAATTTA-ATATATTGATATTTATATCATTTTACGTTTCTCGTTCAGCTTTTTTGTAC-AAAGTTGGCATTATAAAAAAGCATTGCTCATCAATTTGTTGCAACGAACA-GGTCACTATCAGTCAAAATAAAATCATTATTTG-3'

attP2: 5'-CAAATAATGATTTTATTTTGACTGATAGTGACCTGTTTCGTTGCAACAAAT-TGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGCTGAAC-GAGAAACGTAAAATGATATAAATATCAATATATTAAATTAGATTTTGCAT-AAAAACAGACTACATAATACTGTAAACACAACATATCCAGTCACTATGA-ATCAACTACTTAGATGGTATTAGTGACCTGTA-3'

attR1: 5'-ACAAGTTTGTACAAAAAAGCTGAACGAGAAACGTAAAATGATATAAA-TATCAATATATTAAATTAGATTTTGCATAAAAAACAGACTACATAATAC-TGTAAACACAACATATCCAGTCACTATG-3'

attR2: 5'-GCAGGTCGACCATAGTGACTGGATATGTTGTGTTTTACAGTATTAT-GTAGTCTGTTTTTTATGCAAAATCTAATTTAATATATTGATATTT-ATATCATTTTACGTTTCTCGTTCAGCTTTCTTGTACAAAGTGGT-3'

attL1: 5'-CAAATAATGATTTTATTTTGACTGATAGTGACCTGTTTCGTTGCAAC-AAATTGATAAGCAATGCTTTTTTATAATGCCAACTTTGTACAAAAAA-GCAGGCT-3'

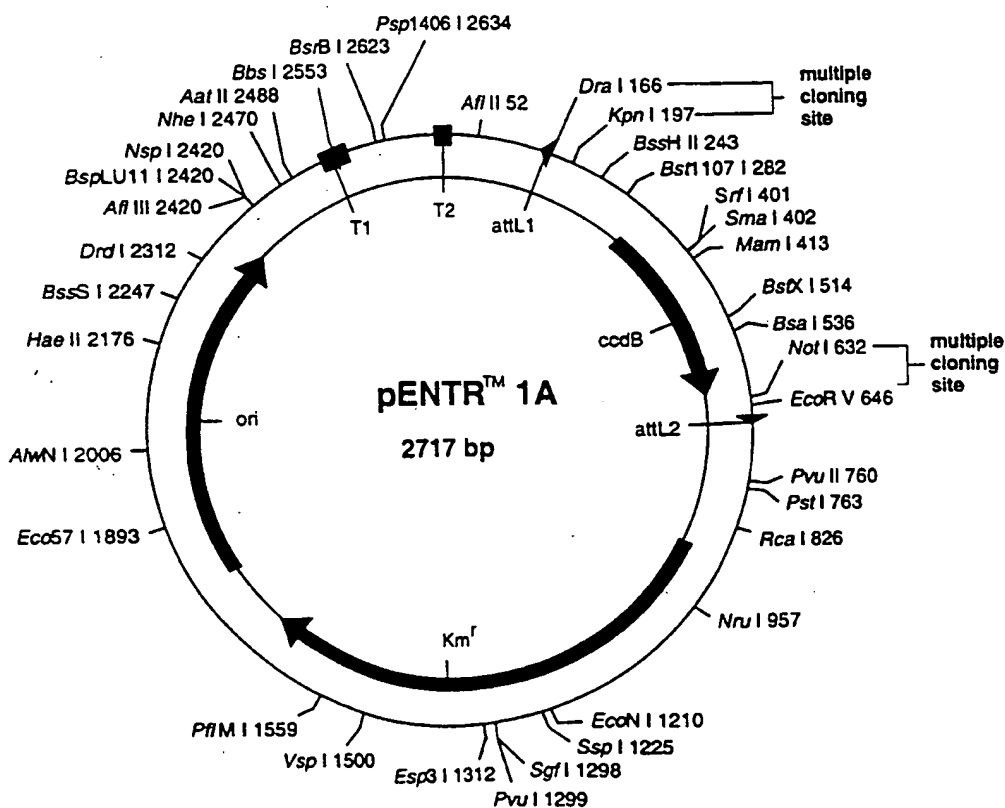
attL2: 5'-CAAATAATGATTTTATTTTGACTGATAGTGACCTGTTTCGTTGCAACAA-ATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGCTGGGT-3'

Figure 9

Figure 10A: Cloning sites of the Entry Vector pENTRTM 1A (reading frame A)

^{Dra I} ^{Xmn I} ^{Sal I} ^{BamH I} ^{Kpn I} ^{EcoR I}
 ACT TTG TAC AAA AAA GCA GGC TTT AAA GGA ACC AAT TCA GTC GAC TGG ATC CGG TAC CGA ATT C
 TGA AAC ATG TTT TTT CGT CCG AAA TTT CCT TGG TTA AGT CAG CTG ACC TAG GCC ATG GCT TAA G
 thr leu tyr lys lys ala gly phe lys gly thr asn ser val asp trp ile arg tyr arg ile

^{EcoR I} ^{Not I} ^{Xho I} ^{EcoR V}
 --- ccdB gene --- G AAT TCG CCG CCG CAC TCG AGA TAT CTA GAC CCA GCT TTC TTG TAC AAA
 C TTA AGC GCC GGC GTG AGC TCT ATA GAT CTG GGT CGA AAG AAC ATG TTT



11/240

pENTR1A 2717 bp

<u>Base Nos.</u>	<u>Gene Encoded</u>
67..166	attL1
321..626	ccdB
655..754	attL2
877..1686	KmR
1791..2364	ori

1	CTGACGGATG	GCCTTTTTCG	GTTTCTACAA	ACTCTTCCTG	TTAGTTAGTT	ACTTAAGCTC
61	GGGCCCCAAA	TAATGATTTT	ATTTTGACTG	ATAGTGACCT	GTTTCGTTGCA	ACAAATTGAT
121	AAGCAATGCT	TTTTTATAAT	GCCAACTTTG	TACAAAAAAG	CAGGCTTTAA	AGGAACCAAT
181	TCAGTCGACT	GGATCCGGTA	CCGAATTCGC	TTACTAAAAG	CCAGATAACA	GTATGCGTAT
241	TTGCGCGCTG	ATTTTTCGCG	TATAAGAAATA	TATACTGATA	TGTATACCCG	AAGTATGTCA
301	AAAAGAGGTG	TGCTTCTAGA	ATGCAGTTTA	AGGTTTACAC	CTATAAAAGA	GAGAGCCGTT
361	ATCGTCTGTT	TGTGGATGTA	CAGAGTGATA	TTATTGACAC	GCCCCGGCGA	CGGATAGTGA
421	TCCCCCTGGC	CAGTGCACGT	CTGCTGTGAG	ATAAAGTCTC	CCGTGAACCT	TACCCGGTGG
481	TGCATATCGG	GGATGAAAGC	TGGCGCATGA	TGACCACCGA	TATGGCCAGT	GTGCCGGTCT
541	CCGTTATCGG	GGAAGAAGTG	GCTGATCTCA	GCCACCGCGA	AAATGACATC	AAAAACGCCA
601	TTAACCTGAT	GTTCTGGGGA	ATATAGAATT	CGCGGCCGCA	CTCGAGATAT	CTAGACCCAG
661	CTTCTTGTA	CAAAGTTGGC	ATTATAAGAA	AGCATTGCTT	ATCAATTTGT	TGCAACGAAC
721	AGGTCACAT	CAGTCAAAAT	AAAATCATTA	TTTGCCATCC	AGCTGCAGCT	CTGGCCCCGTG
781	TCTCAAAATC	TCTGATGTTA	CATTGCACAA	GATAAAAATA	TATCATCATG	AACAATAAAA
841	CTGTCTGCTT	ACATAAACAG	TAATACAAGG	GGTGTATGA	GCCATATTCA	ACGGGAAACG
901	TCGAGGCCGC	GATTAAATTC	CAACATGGAT	GCTGATTTAT	ATGGGTATAA	ATGGGCTCGC
961	GATAATGTCG	GGCAATCAGG	TGCGACAATC	TATCGCTTGT	ATGGGAAGCC	CGATGCGCCA
1021	GAGTTTTC	TGAAACATGG	CAAAGGTAGC	GTTGCCAATG	ATGTTACAGA	TGAGATGGTC
1081	AGACTAAACT	GGCTGACGGA	ATTTATGCCT	CTTCCGACCA	TCAAGCATTT	TATCCGTACT
1141	CCTGATGATG	CATGGTTACT	CACCACTGCG	ATCCCCGGAA	AAACAGCATT	CCAGGTATTA
1201	GAAGAATATC	CTGATTCAGG	TGAAAATATT	GTTGATGCGC	TGGCAGTGTC	CCTGCGCCGG
1261	TTGCATTGCA	TTCTGTTTTC	TAATTGTCTT	TTTAACAGCG	ATCGCGTATT	TCGTCTCGCT
1321	CAGGCGCAAT	CACGAATGAA	TAACGGTTTG	GTTGATGCGA	GTGATTTTGA	TGACGAGCGT
1381	AATGGCTGGC	CTGTTGAACA	AGTCTGAAA	GAAATGCATA	AACTTTTGCC	ATTCTCACCG
1441	GATTCACTCG	TCACTCATGG	TGATTCTCTA	CTTGATAACC	TTATTTTGA	CGAGGGGAAA
1501	TTAATAGGTT	GTATTGATGT	TGGACGAGTC	GGAAATCGCAG	ACCGATACCA	GGATCTTGCC
1561	ATCCTATGGA	ACTGCCTCGG	TGAGTTTCTT	CCTTCATTAC	AGAAACGGCT	TTTTCAAAAA
1621	TATGGTATTG	ATAATCCTGA	TATGAATAAA	TTGCAGTTTC	ATTTGATGCT	CGATGAGTTT
1681	TTCTAATCAG	AATTGGTTAA	TTGGTTGTAA	CATTATTTCAG	ATTGGGCCCC	GTTCCACTGA
1741	GCGTCAGACC	CCGTAGAAAA	GATCAAAGGA	TCTTCTTGAG	ATCCTTTTTT	TCTGCGCGTA
1801	ATCTGTGCT	TGCAAAACAA	AAAACCACCG	CTACCAGCGG	TGGTTTGTTT	GCCGGATCAA
1861	GAGCTACCAA	CTCTTTTTC	GAAGGTAAC	GGCTTCAGCA	GAGCGCAGAT	ACCAAATACT
1921	GTTCTTCTAG	TGTAGCCGTA	GTTAGGCCAC	CACTTCAAGA	ACTCTGTAGC	ACCGCCTACA
1981	TACCTCGCTC	TGCTAATCCT	GTTACCAGTG	GCTGCTGCCA	GTGGCGATAA	GTCGTGTCTT
2041	ACCGGGTTGG	ACTCAAGACG	ATAGTTACCG	GATAAGGCGC	AGCGGTCGGG	CTGAACGGGG
2101	GGTTCGTGCA	CACAGCCCAG	CTTGGAGCGA	ACGACCTACA	CCGAACCTGAG	ATACCTACAG
2161	CGTGAGCTAT	GAGAAAGCGC	CACGCTTCCC	GAAGGGAGAA	AGGCGGACAG	GTATCCGGTA
2221	AGCGGCAGGG	TCGGAACAGG	AGAGCGCACG	AGGGAGCTTC	CAGGGGGAAA	CGCCTGGTAT
2281	CTTTATAGTC	CTGTCCGGTT	TCGCCACCTC	TGACTTGAGC	GTCGATTTTT	GTGATGCTCG
2341	TCAGGGGGGC	GGAGCCTATG	GA AAAACGCC	AGCAACGCGG	CCTTTTTTACG	GTTCTGGGCC
2401	TTTTGCTGGC	CTTTTGCTCA	CATGTTCTTT	CCTGCGTTAT	CCCCTGATTG	TGTGGATAAC
2461	CGTATTACCG	CTAGCATGGA	TCTCGGGGAC	GTCTAACTAC	TAAGCGAGAG	TAGGGAACCTG
2521	CCAGGCATCA	AATAAAACGA	AAGGCTCAGT	CGGAAGACTG	GGCCTTTCGT	TTTATCTGTT
2581	GTTTGTGCGT	GAACGCTCTC	CTGAGTAGGA	CAAAATCCGCC	GGGAGCGGAT	TTGAACGTTG
2641	TGAAGCAACG	GCCCCGAGGG	TGGCGGGCAG	GACGCCCCGC	ATAAACTGCC	AGGCATCAAA
2701	CTAAGCAGAA	GGCCATC				

FIGURE 10B

Figure 11A: Cloning Sites of the Entry Vector pENTR2B (reading frame B)

Int	attL1	EheI	XmnI	Sall	BamHI
TTG TAC AAA AAA GCA GGC TGG	CAC CGG AAC CAA TTC AGT CGA CTG	GAT CCG			
AAC ATG TTT TTT CGT CCG ACC	GCG GCC TTG GTT AAG TCA GCT	GAC CTA GGC			
Leu Tyr Lys Lys Ala Gly Trp	Arg Arg Asn Gln Phe Ser Arg	Leu Asp Pro			

KpnI	EcoRI	EcoRI	NotI	XhoI	EcoRV	XbaI
GTA GCG AAT TC- ccdB --G	AAT TCG CCG CCG CAC	TCG AGA TAT	CTA GAC CCA			
CAT GGC TTA AG	C TTA AGC GCC GGC	GTG AGC TCT	ATA GAT CTG GGT			
Val Pro Asn	Asn Ser Arg Pro	His Ser Arg Tyr	Leu Asp Pro			

Int	attL2
GCT TTC TTG TAC AAA G	
CGA AAG AAC ATG TTT C	
Ala Phe Leu Tyr Lys	

pENTR2B 2718 bp

<u>Location (Base Nos.)</u>	<u>Gene Encoded</u>
67..166	attL1
322..627	ccdB
656..755	attL2
878..1687	KmR
1792..2365	ori

```

1 CTGACGGATG GCCTTTTTCG GTTCTACAA ACTCTTCCTG TTAGTTAGTT ACTTAAGCTC
61 GGGCCCCAAA TAATGATTTT ATTTTGACTG ATAGTGACCT GTTCGTTGCA ACAAATTGAT
121 AAGCAATGCT TTTTATAAT GCCAACTTTG TACAAAAAAG CAGGCTGGCG CCGGAACCAA
181 TTCAGTCGAC TGGATCCGGT ACCGAATTCG CTTACTAAAA GCCAGATAAC AGTATGCGTA
241 TTGCGCGCT GATTTTTCG GTATAAGAAT ATATACTGAT ATGTATACCC GAAGTATGTC
301 AAAAAGAGGT GTGCTTCTAG AATGCAGTTT AAGGTTTACA CCTATAAAAG AGAGAGCCGT
361 TATCGTCTGT TTGTGGATGT ACAGAGTGAT ATTATTGACA CGCCCGGGCG ACGGATGGTG
421 ATCCCCCTGG CCAGTGCACG TCTGCTGTCA GATAAAGTCT CCCGTGAAC TTACCCGGTG
481 GTGCATATCG GGGATGAAAG CTGGCGCATG ATGACCACCG ATATGGCCAG TGTGCCGGTC
541 TCCGTTATCG GGAAGAAGT GGCTGATCTC AGCCACCGCG AAAATGACAT CAAAAACGCC
601 ATTAACCTGA TGTTCTGGGG AATATAGAAT TCGCGGCCGC ACTCGAGATA TCTAGACCCA
661 GCTTCTTGT ACAAAGTTGG CATTATAAGA AAGCATTGCT TATCAATTTG TTGCAACGAA
721 CAGGTCAC TAAGTCAAAA TAAATCATT ATTTGCCATC CAGCTGCAGC TCTGGCCCGT
781 GTCTCAAAAT CTCTGATGTT ACATTGCACA AGATAAAAT ATATCATCAT GAACAATAAA
841 ACTGTCTGCT TACATAAACA GTAATACAAG GGGTGTATG AGCCATATTC AACGGGAAAC
901 GTCGAGGCCG CGATTAAATT CCAACATGGA TGCTGATTTA TATGGGTATA AATGGGCTCG
961 CGATAATGTC GGGCAATCAG GTGCGACAAT CTATCGCTTG TATGGGAAGC CCGATGCGCC
1021 AGAGTTGTTT CTGAAACATG GCAAAGGTAG CGTTGCCAAT GATGTTACAG ATGAGATGGT
1081 CAGACTAAAC TGGCTGACCG AATTATGCC TCTCCGACC ATCAAGCATT TTATCCGTAC
1141 TCCTGATGAT GCATGGTTAC TCACCACTGC GATCCCCGGA AAAACAGCAT TCCAGGTATT
1201 AGAAGAATAT CCTGATTGAG GTGAAAATAT TGTGATGCG CTGGCAGTGT TCCTGCGCCG
1261 GTTGCAATCG ATTCCTGTTT GTAATTGTCC TTTAACAGC GATCGCGTAT TTCGTCTCGC
1321 TCAGGCGCAA TCACGAATGA ATAACGGTTT GGTGATGCG AGTGATTTTG ATGACGAGCG
1381 TAATGGCTGG CCTGTTGAAC AAGTCTGGAA AGAAATGCAT AAACCTTTGC CATTCTCACC
1441 GGATTCAGTC GTCACATCAT GTGATTCTC ACTTGATAAC CTTATTTTGG ACGAGGGGAA
1501 ATTAATAGGT TGTATTGATG TTGGACGAGT CGGAATCGCA GACCGATACC AGGATCTTGC
1561 CATCCTATGG AACTGCCTCG GTGAGTTTTC TCCTTCATTA CAGAAACGGC TTTTCAAAA
1621 ATATGGTATT GATAATCCTG ATATGAATAA ATTGCAGTTT CATTGATGTC TCGATGAGTT
1681 TTTCTAATCA GAATTGGTTA ATTGTTGTA ACATTATTCA GATTGGGCCC CGTTCCACTG
1741 AGCGTCAGAC CCCGTAGAAA AGATCAAAGG ATCTTCTTGA GATCCTTTT TTCTGCGCGT
1801 AATCTGCTGC TTGCAAACAA AAAAACCACC GCTACCAGCG GTGGTTTGTT TGCCGGATCA
1861 AGAGCTACCA ACTCTTTTTC CGAAGGTAAC TGGCTTCAGC AGAGCGCAGA TACCAAATAC
1921 TGTTCTTCTA GTGTAGCCGT AGTTAGGCCA CCACTTCAAG AACTCTGTAG CACCGCCTAC
1981 ATACCTCGCT CTGCTAATCC TGTTACCAGT GGCTGCTGCC AGTGGCGATA AGTCGTGTCT
2041 TACCGGGTTG GACTCAAGAC GATAGTTACC GGATAAGGCG CAGCGGTCGG GCTGAACGGG
2101 GGGTTCGTGC ACACAGCCCA GCTTGAGCG AACGACCTAC ACCGAAGTGA GATACCTACA
2161 GCGTGAGCTA TGAGAAAGCG CCACGCTTCC CGAAGGGAGA AAGGCGGACA GGTATCCGGT
2221 AAGCGGCAGG GTCGGAACAG GAGAGCGCAC GAGGGAGCTT CCAGGGGGAA ACGCCTGGTA
2281 TCTTTATAGT CCTGTGCGGT TTCGCCACCT CTGACTTGAG CGTCGATTTT TGTGATGCTC
2341 GTCAGGGGGG CGGAGCCTAT GGAAAAACGC CAGCAACGCG GCCTTTTTC GGTTCCTGGC
2401 CTTTGTGCTG CTTTGTGCTC ACATGTTCTT TCCTGCGTTA TCCCCTGATT CTGTGGATAA
2461 CCGTATTACC GCTAGCATGG ATCTCGGGA CGTCTAATA CTAAGCGAGA GTAGGGAAC
2521 GCCAGGCATC AAATAAACG AAAGGCTCAG TCGGAAGACT GGGCCTTTCG TTTTATCTGT
2581 TGTTTGTGCG TGAACGCTCT CCTGAGTAGG ACAAATCCGC CGGGAGCGGA TTTGAACGTT
2641 GTGAAGCAAC GGCCCGGAGG GTGGCGGGCA GGACGCCGCG CATAAACTGC CAGGCATCAA
2701 ACTAAGCAGA AGGCCATC

```

FIGURE 11B

Figure 2A: Cloning Sites of the Entry Vector pENTR3C (reading frame C) ...

Int	attL1		DraI		XmnI		SalI		BamHI								
TTG	TAC	AAA	AAA	GCA	GGC	TCT	TTA	AAG	GAA	CCA	ATT	CAG	TCG	ACT	GGA	TCC	GGT
AAC	ATG	TTT	TTT	CGT	CCG	AGA	AAT	TTC	CTT	GGT	TAA	GTC	AGC	TGA	CCT	AGG	CCA
Leu	Tyr	Lys	Lys	Ala	Gly	Ser	Leu	Lys	Glu	Pro	Ile	Gln	Ser	Thr	Gly	Ser	Gly

KpnI	EcoRI		PvuI		EcoRI		NotI		XhoI		EcoRV	XbaI			
ACG	GAA	TTC	GAT	CGC--	ccdB	--G	AAT	TCG	CGG	CCG	CAC	TCG	AGA	TAT	CTA
TGG	CTT	AAG	CTA	GCG		C	TTA	AGC	GCC	GGC	GTG	AGC	TCT	ATA	GAT
Thr	Glu	Phe					Asn	Ser	Arg	Pro	His	Ser	Arg	Tyr	Leu

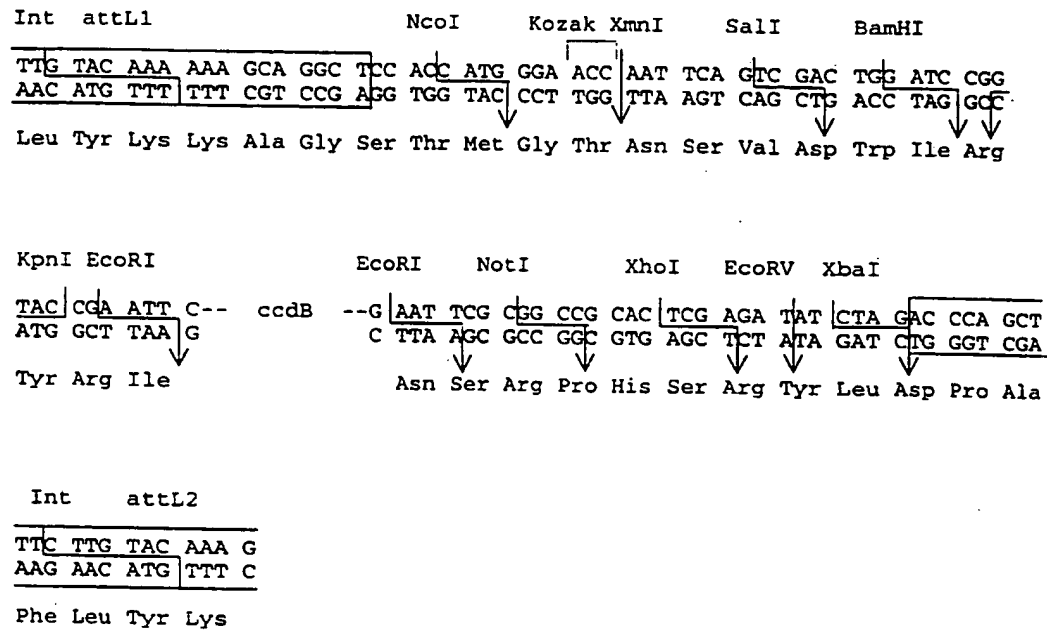
attL2	Int						
GAC	CCA	GCT	TTC	TTG	TAC	AAA	G
CTG	GGT	CGA	AAG	AAC	ATG	TTT	C
Asp	Pro	Ala	Phe	Leu	Tyr	Lys	

15/240

pENTR3C 2723 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
67..166		attL1
327..632		ccdB
661..760		attL2
883..1692		KmR
1797..2370		ori
1	CTGACGGATG GCCTTTTTGC GTTTCTACAA ACTCTTCCTG TTAGTTAGTT ACTTAAGCTC	
61	GGGCCCCAAA TAATGATTTT ATTTTGACTG ATAGTGACCT GTTCGTTGCA ACAAATTGAT	
121	AAGCAATGCT TTTTATAAT GCCAACTTTG TACAAAAAAG CAGGCTCTTT AAAGGAACCA	
181	ATTCACTCGA CTGGATCCGG TACCGAATTC GATCGCTTAC TAAAAGCCAG ATAACAGTAT	
241	GCGTATTTGC GCGCTGATTT TTGCGGTATA AGAATATATA CTGATATGTA TACCCGAAGT	
301	ATGTCAAAAA GAGGTGTGCT TCTAGAATGC AGTTTAAGGT TTACACCTAT AAAAGAGAGA	
361	GCCGTTATCG TCTGTTTGTG GATGTACAGA GTGATATTAT TGACACGCCG GGGCGACGGA	
421	TGGTGATCCC CCTGGCCAGT GCACGTCTGC TGTCAGATAA AGTCTCCCGT GAACTTTACC	
481	CGGTGGTGCA TATCGGGGAT GAAAGCTGGC GCATGATGAC CACCGATATG GCCAGTGTGC	
541	CGGTCTCCGT TATCGGGGAA GAAGTGGCTG ATCTCAGCCA CCGCGAAAAA GACATCAAAA	
601	ACGCCATTAA CCTGATGTTC TGGGGAATAT AGAATTCGCG GCCGCACTCG AGATATCTAG	
661	ACCCAGCTTT CTTGTACAAA GTTGGCATTG TAAGAAAGCA TTGCTTATCA ATTGTTGCA	
721	ACGAACAGGT CACTATCAGT CAAAATAAAA TCATTATTTG CCATCCAGCT GCAGCTCTGG	
781	CCCGTGTCTC AAAATCTCTG ATGTTACATT GCACAAGATA AAAATATATC ATCATGAACA	
841	ATAAACTGT CTGCTTACAT AAACAGTAAT ACAAGGGGTG TTATGAGCCA TATTCAACGG	
901	GAAACGTCGA GGCCGCGATT AAATCCAAC ATGGATGCTG ATTTATATGG GTATAAATGG	
961	GCTCGCGATA ATGTCGGGCA ATCAGGTGCG ACATCTATC GCTTGTATGG GAAGCCCGAT	
1021	GCGCCAGAGT TGTTTCTGAA ACATGGCAAA GGTAGCGTTG CCAATGATGT TACAGATGAG	
1081	ATGGTCAGAC TAAACTGGCT GACGGAATTT ATGCCTCTTC CGACCATCAA GCATTTTATC	
1141	CGTACTCCTG ATGATGCATG GTTACTCACC ACTGCGATCC CCGGAAAAAC AGCATTTCCAG	
1201	GTATTAGAAG AATATCCTGA TTCAGGTGAA AATATTGTTG ATGCGCTGGC AGTGTTCCTG	
1261	CGCCGGTTGC ATTCGATTCC TGTTTGTAAT TGTCCTTTTA ACAGCGATCG CGTATTTCTG	
1321	CTCGCTCAGG CGCAATCACG AATGAATAAC GGTTTGTTG ATGCGAGTGA TTTTGATGAC	
1381	GAGCGTAATG GCTGGCCTGT TGAACAAGTC TGGAAGAAA TGCATAAACT TTTGCCATTG	
1441	TCACCGGATT CAGTCGTCAC TCATGGTGAT TTCTCACTTG ATAACCTTAT TTTTGACGAG	
1501	GGGAAATTAA TAGGTTGTAT TGATGTTGGA CGAGTCGGAA TCGCAGACCG ATACCAGGAT	
1561	CTTGCCATCC TATGGAACG CCTCGGTGAG TTTTCTCCTT CATTACAGAA ACGGCTTTTT	
1621	CAAAAATATG GTATTGATAA TCCTGATATG AATAAATTGC AGTTTCATTT GATGCTCGAT	
1681	GAGTTTTTCT AATCAGAATT GGTAAATTGG TTGTAACATT ATTCAGATTG GGCCCGGTTT	
1741	CACTGAGCGT CAGACCCCGT AGAAAAGATC AAAGGATCTT CTGAGATCC TTTTCTCTG	
1801	CGCGTAATCT GCTGCTTGCA AACAAAAAAA CCACCGCTAC CAGCGGTGGT TTGTTTGCCG	
1861	GATCAAGAGC TACCAACTCT TTTTCCGAAG GTAACCTGGT TCAGCAGAGC GCAGATACCA	
1921	AATACTGTTT TTCTAGTGTA GCCGTAGTTA GGCCACCACT TCAAGAACTC TGTAGCACCG	
1981	CCTACATACC TCGCTCTGCT AATCCTGTGA CCAGTGGCTG CTGCCAGTGG CGATAAGTCG	
2041	TGTCTTACCG GGTGGACTC AAGACGATAG TTACCGGATA AGGCGCAGCG GTCGGGCTGA	
2101	ACGGGGGGTT CGTGACACA GCCCAGCTTG GAGCGAACGA CCTACACCGA ACTGAGATAC	
2161	CTACAGCGTG AGCTATGAGA AAGCGCCACG CTTCCCGAAG GGAGAAAGGC GGACAGGTAT	
2221	CCGGTAAGCG GCAGGGTCGG AACAGGAGAG CGCACGAGGG AGCTTCCAGG GGGAAACGCC	
2281	TGGTATCTTT ATAGTCCTGT CGGGTTTCGC CACCTCTGAC TTGAGCGTCG ATTTTGTGTA	
2341	TGCTCGTCAG GGGGGCGGAG CCTATGGAAA AACGCCAGCA ACGCGGCCTT TTTACGGTTC	
2401	CTGGCCCTTT GCTGGCCTTT TGCTCACATG TTCTTCTCTG CGTTATCCCC TGATTCTGTG	
2461	GATAACCGTA TTACCGCTAG CATGGATCTC GGGGACGTCT AACTACTAAG CGAGAGTAGG	
2521	GAACTGCCAG GCATCAAATA AAACGAAAGG CTCAGTCGGA AGACTGGGCC TTTCTGTTTA	
2581	TCTGTTGTTT GTCGGTGAAC GCTCTCCTGA GTAGGACAAA TCCGCCGGGA GCGGATTTGA	
2641	ACGTTGTGAA GCAACGGCCC GGAGGGGTGGC GGGCAGGACG CCCGCCATAA ACTGCCAGGC	
2701	ATCAAATAA GCAGAAGGCC ATC	

FIGURE 12B

Figure 13A: Cloning Sites of the Entry Vector pENTR4 :

17/240

pENTR4 2720 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
67..166		attL1
324..629		ccdB
658..757		attL2
880..1689		KmR
1794..2367		ori
1	CTGACGGATG GCCTTTTTCG GTTTCTACAA ACTCTTCCTG TTAGTTAGTT ACTTAAGCTC	
61	GGGCCCCAAA TAATGATTTT ATTTTGACTG ATAGTGACCT GTTCGTTGCA ACAAATTGAT	
121	AAGCAATGCT TTTTATAAT GCCAACTTTG TACAAAAAAG CAGGCTCCAC CATGGGAACC	
181	AATTCAGTCG ACTGGATCCG GTACCGAATT CGCTTACTAA AAGCCAGATA ACAGTATGCG	
241	TATTTGCGCG CTGATTTTTG CGGTATAAGA ATATATACTG ATATGTATAC CCGAAGTATG	
301	TCAAAAAGAG GTGTGCTTCT AGAATGCAGT TTAAGGTTTA CACCTATAAA AGAGAGAGCC	
361	GTTATCGTCT GTTGTGGAT GTACAGAGTG ATATTATTGA CACGCCCGGG CGACGGATGG	
421	TGATCCCCCT GGCCAGTGCA CGTCTGCTGT CAGATAAAGT CTCCCGTGAA CTTTACCCGG	
481	TGGTGCATAT CGGGGATGAA AGCTGGCGCA TGATGACCAC CGATATGGCC AGTGTGCCGG	
541	TCTCCGTTAT CGGGGAAGAA GTGGCTGATC TCAGCCACCG CGAAAATGAC ATCAAAAACG	
601	CCATTAACTT GATGTTCTGG GGAATATAGA ATTGCGGGCC GCACTCGAGA TATCTAGACC	
661	CAGCTTTCTT GTACAAAGTT GGCAATTATA GAAAGCATTG CTTATCAATT TGTGCAACG	
721	AACAGGTCAC TATCAGTCAA AATAAAATCA TTATTTGCCA TCCAGCTGCA GCTCTGGCCC	
781	GTGTCTCAAA ATCTCTGATG TTACATTGCA CAAGATAAAA ATATATCATC ATGAACAATA	
841	AAACTGTCTG CTTACATAAA CAGTAATACA AGGGGTGTTA TGAGCCATAT TCAACGGGAA	
901	ACGTCGAGGC CGCGATTAAA TTCCAACATG GATGCTGATT TATATGGGTA TAAATGGGCT	
961	CGCGATAATG TCGGGCAATC AGGTGCGACA ATCTATCGCT TGTATGGGAA GCCCGATGCG	
1021	CCAGAGTTGT TTCTGAAACA TGGCAAAGGT AGCGTTGCCA ATGATGTTAC AGATGAGATG	
1081	GTCAGACTAA ACTGGCTGAC GGAATTTATG CCTCTCCGA CCATCAAGCA TTTTATCCGT	
1141	ACTCCTGGTG ATGCATGGTT ACTCACCACG GCGATCCCCG GAAAAACAGC ATTCCAGGTA	
1201	TTAGAAGAAT ATCCTGATTC AGGTGAAAAT ATTGTTGATG CGCTGGCAGT GTTCTGCGC	
1261	CGGTTGCATT CGATTCTCTG TTGTAATTGT CCTTTAACA GCGATCGCGT ATTTCTGCTC	
1321	GCTCAGGCGC AATCACGAAT GAATAACGGT TTGGTTGATG CGAGTGATTT TGATGACGAG	
1381	CGTAATGGCT GGCCTGTTGA ACAAGTCTGG AAAGAAATGC ATAACTTTT GCCATTCTCA	
1441	CCGGATTTCG TCGTCACTCA TGGTGATTTT TCACTTGATA ACCTTATTTT TGACGAGGGG	
1501	AAATTAATAG GTTGATTGTA GTTTGGACGA GTCGGAATCG CAGACCGATA CCAGGATCTT	
1561	GCCATCCTAT GGAAGTGCCT CGGTGAGTTT TCTCCTTCAT TACAGAAACG GCTTTTTTCAA	
1621	AAATATGGTA TTGATAATCC TGATATGAAT AAATTGCAGT TTCATTTGAT GCTCGATGAG	
1681	TTTTTCTAAT CAGAAATTGGT TAATTGGTTG TAACATTATT CAGATTGGGC CCCGTTCAC	
1741	TGAGCGTCAG ACCCCGTAGA AAAGATCAAA GGATCTTCTT GAGATCCTTT TTTTCTGCGC	
1801	GTAATCTGCT GCTTGCAAAC AAAAAACCA CCGTACCAG CGGTGGTTTG TTTGCCGGAT	
1861	CAAGAGCTAC CAACTCTTTT TCCGAAGGTA ACTGGCTTCA GCAGAGCGCA GATACCAAAT	
1921	ACTGTTCTTC TAGTGTAGCC GTAGTTAGGC CACCACTTCA AGAACTCTGT AGCACCGCCT	
1981	ACATACCTCG CTCTGCTAAT CCTGTTACCA GTGGCTGCTG CCAGTGGCGA TAAGTCGTGT	
2041	CTTACCGGGT TGGACTCAAG ACGATAGTTA CCGGATAAGG CGCAGCGGTC GGGCTGAACG	
2101	GGGGGTTTCG GCACACAGCC CAGCTTGAG CGAACGACCT ACACCGAACT GAGATACCTA	
2161	CAGCGTGAGC TATGAGAAAG CGCCACGCTT CCCGAAGGGA GAAAGGCGGA CAGGTATCCG	
2221	GTAAGCGGCA GGGTCGGAAC AGGAGAGCGC ACGAGGGAGC TTCCAGGGG AAACGCCTGG	
2281	TATCTTTATA GTCCTGTCGG GTTTCGCCAC CTCGACTTG AGCGTCGATT TTTGTGATGC	
2341	TCGTGAGGGG GCGGAGCCT ATGGAAAAAC GCCAGCAACG CGGCCTTTTT ACGGTTCCTG	
2401	GCCTTTTGCT GGCCTTTTGC TCACATGTTT TTTCTGCGT TATCCCCTGA TTCTGTGGAT	
2461	AACCGTATTA CCGCTAGCAT GGATCTCGGG GACGTCTAAC TACTAAGCGA GAGTAGGGAA	
2521	CTGCCAGGCA TCAATAAAAA CGAAAGGCTC AGTCGGAAGA CTGGGCCTTT CGTTTTATCT	
2581	GTTGTTTGTC GGTGAACGCT CTCCTGAGTA GGACAAATCC GCCGGGAGCG GATTTGAACG	
2641	TTGTGAAGCA ACGGCCGGA GGGTGCGGG CAGGACGCCC GCCATAAACT GCCAGGCATC	
2701	AAACTAAGCA GAAGGCCATC	

FIGURE 13B

19/260

pENTR5 2720 bp

Location (Base Nos.)	Gene Encoded
67..166	attL1
324..629	ccdB
658..757	attL2
880..1689	KmR
1794..2367	ori

```

1 CTGACGGATG GCCTTTTTCG GTTCTACAA ACTCTTCCTG TTAGTTAGTT ACTTAAGCTC
61 GGGCCCCAAA TAATGATTTT ATTTTGACTG ATAGTGACCT GTTCGTTGCA ACAAATTGAT
121 AAGCAATGCT TTTTATAAT GCCAACTTTG TACAAAAAAG CAGGCTTTCA TATGGGAACC
181 AATTCAGTCG ACTGGATCCG GTACCGAATT CGCTTACTAA AAGCCAGATA ACAGTATGCG
241 TATTTGCGCG CTGATTTTTCG CGGTATAAGA ATATATACTG ATATGTATAC CCGAAGTATG
301 TCAAAAAGAG GTGTGCTTCT AGAATGCAGT TTAAGGTTTA CACCTATAAA AGAGAGAGCC
361 GTTATCGTCT GTTTGTGGAT GTACAGAGTG ATATTATTGA CACGCCCGGG CGACGGATGG
421 TGATCCCCCT GGCCAGTGCA CGTCTGCTGT CAGATAAAGT CTCCCGTGAA CTTTACCCGG
481 TGGTGCATAT CGGGGATGAA AGCTGGCGCA TGATGACCAC CGATATGGCC AGTGTGCCGG
541 TCTCCGTTAT CGGGGAAGAA GTGGCTGATC TCAGCCACCG CGAAAATGAC ATCAAAAACG
601 CCATTAACCT GATGTTCTGG GGAATATAGA ATTCGCGGCC GCACTCGAGA TATCTAGACC
661 CAGCTTTCTT GTACAAAGTT GGCATTATAA GAAAGCATTG CTTATCAATT TGTTCGAACG
721 AACAGGTCAC TATCAGTCAA AATAAAATCA TTATTTGCCA TCCAGCTGCA GCTCTGGCCC
781 GTGTCTCAA ATCTCTGATG TTACATTGCA CAAGATAAAA ATATATCATC ATGAACAATA
841 AAAGTGTCTG CTTACATAAA CAGTAATACA AGGGGTGTTA TGAGCCATAT TCAACGGGAA
901 ACGTCGAGGC CGCGATTAAA TTCCAACATG GATGCTGATT TATATGGGTA TAAATGGGCT
961 CGCGATAATG TCGGGCAATC AGGTGCGACA ATCTATCGCT TGTATGGGAA GCCCGATGCG
1021 CCAGAGTTGT TTCTGAAACA TGGCAAAGGT AGCGTTGCCA ATGATGTTAC AGATGAGATG
1081 GTCAGACTAA ACTGGCTGAC GGAATTTATG CCTCTCCGA CCATCAAGCA TTTTATCCGT
1141 ACTCCTGATG ATGCATGGTT ACTCACCAC GCGATCCCCG GAAAAACAGC ATTCCAGGTA
1201 TTAGAAGAAT ATCCTGATTC AGGTGAAAAT ATTGTTGATG CGCTGGCAGT GTTCCTGCGC
1261 CGGTTGCATT CGATTCCTGT TTGTAATTGT CCTTTTAACA GCGATCGCGT ATTTCTGCTC
1321 GCTCAGGCGC AATCACGAAT GAATAACGGT TTGGTTGATG CGAGTGATTT TGATGACGAG
1381 CGTAATGGCT GGCCTGTTGA ACAAGTCTGG AAAGAAATGC ATAAACTTTT GCCATTCTCA
1441 CCGGATTCAG TCGTCACTCA TGGTGATTTT TCACTTGATA ACCTTATTTT TGACGAGGGG
1501 AAATTAATAG GTTGATTTGA GTGTGGACGA GTCGGAATCG CAGACCGATA CCAGGATCTT
1561 GCCATCCTAT GGAAGTGCCT CGGTGAGTTT TCTCCTTCAT TACAGAAACG GCTTTTTCAA
1621 AAATATGGTA TTGATAATCC TGATATGAAT AAATTGCAGT TTCATTTGAT GCTCGATGAG
1681 TTTTCTTAAT CAGAATTGGT TAATTGGTTG TAACATTATT CAGATTGGGC CCCGTTCCAC
1741 TGAGCGTCAG ACCCCGTAGA AAAGATCAAA GGATCTTCTT GAGATCCTTT TTTTCTGCGC
1801 GTAATCTGCT GCTTGCAAAC AAAAAAACCA CCGTACCAG CGGTGGTTTG TTTGCCGGAT
1861 CAAGAGCTAC CAACTCTTTT TCCGAAGGTA ACTGGCTTCA GCAGAGCGCA GATACCAAT
1921 ACTGTTCTTC TAGTGTAGCC GTAGTTAGGC CACCACTTCA AGAAGTCTGT AGCACCCTCT
1981 ACATACCTCG CTCTGCTAAT CCTGTTACCA GTGGCTGCTG CCAGTGGCGA TAAGTCGTGT
2041 CTTACCGGGT TGGACTCAAG ACGATAGTTA CCGGATAAGG CGCAGCGGTC GGGCTGAACG
2101 GGGGGTTCGT GCACACAGCC CAGCTTGGAG CGAACGACCT ACACCGAAGT GAGATACCTA
2161 CAGCGTGAGC TATGAGAAAG CGCCACGCTT CCCGAAGGGA GAAAGGCGGA CAGGTATCCG
2221 GTAAGCGGCA GGGTCGGAAC AGGAGAGCGC ACGAGGGAGC TTCCAGGGGG AAACGCCTGG
2281 TATCTTTATA GTCCTGTCGG GTTTCGCCAC CTCTGACTTG AGCGTCGATT TTTGTGATGC
2341 TCCTCAGGGG GCGGAGCCCT ATGGAAAAAC GCCAGCAACG CGGCCTTTT ACGGTTCCTG
2401 GCCTTTTGCT GGCCTTTTGC TCACATGTTT TTTCTGCGT TATCCCTGTA TTCTGTGGAT
2461 AACCGTATTA CCGCTAGCAT GGATCTCGGG GACGTCTAAC TACTAAGCGA GAGTAGGGAA
2521 CTGCCAGGCA TCGAATAAAA CGAAAGGCTC AGTCGGAAGA CTGGGCCCTT CGTTTTATCT
2581 GTTGTGTTGC GGTGAACGCT CTCCTGAGTA GGACAAATCC GCCGGGAGCG GATTTGAACG
2641 TTGTGAAGCA ACGGCCCGGA GGGTGGCGGG CAGGACGCCC GCCATAAATC GCCAGGCATC
2701 AAATAAGCA GAAGGCCATC

```

Figure 14B

20/240

Figure 1A: Cloning sites of the Entry Vector pENTR6

Int attL1 Sph I Kpn I Xmn I Sma I
~~--- tgg tac aaa aaa gca ggc tgc atg cga acc aat tca gtc~~
~~--- aac atg tct ttt cgt ccg acc tac gct tgg tta agt cag~~
 Leu Tyr Lys Lys Ala Gly Cys Met Arg Thr Asn Ser Val

BamH I Kpn I EpeR I EcoR I
 gac tgg atc cgg tac cga att cgc --- Death --- aga att cgc
 cgg acc tag gcp atg gct taa gcg --- (codB) --- tct taa gcg
 Asp Trp Ile Arg Tyr Arg Ile

Not Xho I EcoR I Xba I Int attL2
 ggc cgc act cga gat atc tag acc cag att tgc tgt aga aag ---
 ccg gcg tga gct cta tag atc tgg gtc gaa aga aca tgt ttc ---

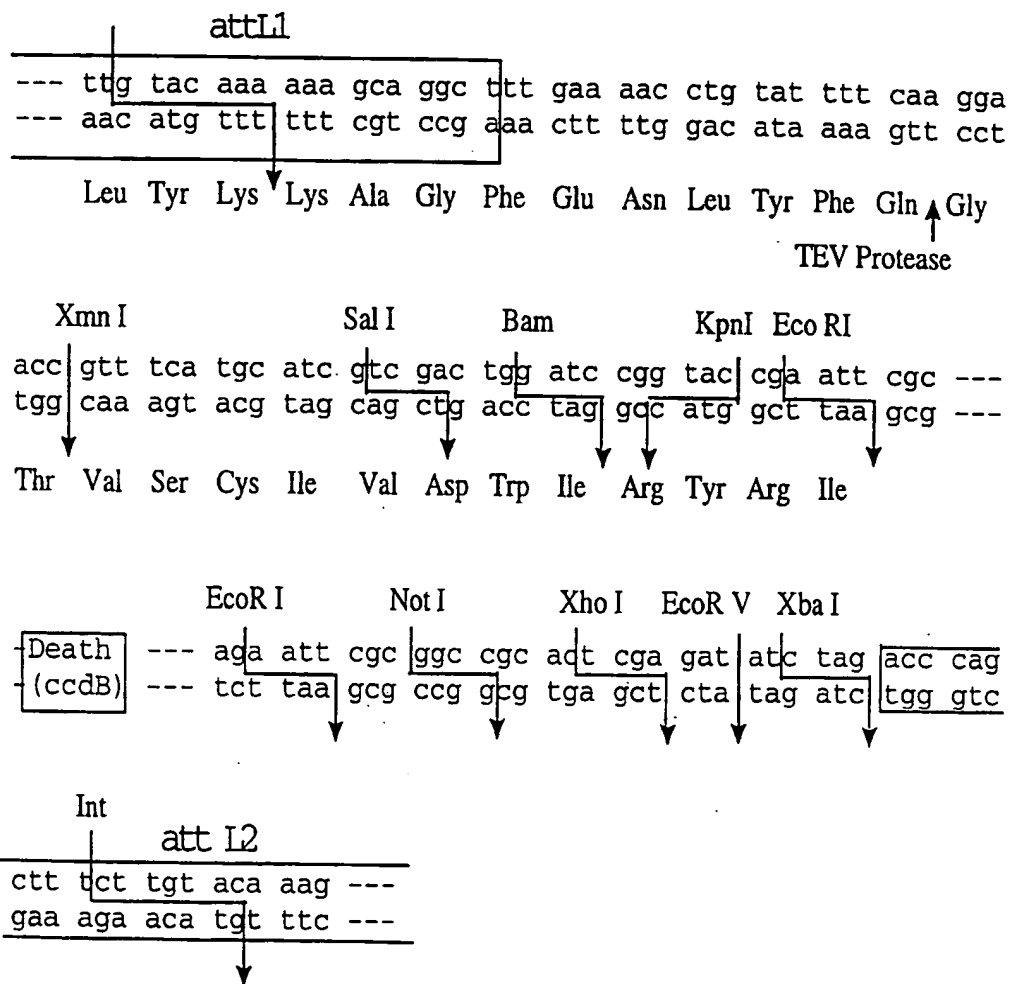
21/240

pENTR6 2717 bp

<u>Location (Base Nos.)</u>				<u>Gene Encoded</u>		
67..166				attL1		
321..626				ccdB		
655..754				attL2		
877..1686				KmR		
1791..2364				ori		
1	CTGACGGATG	GCCTTTTTTGC	GTTTCTACAA	ACTCTTCCTG	TTAGTTAGTT	ACTTAAGCTC
61	GGGCCCCAAA	TAATGATTTT	ATTTTGACTG	ATAGTGACCT	GTTCTGTTGCA	ACAAATTGAT
121	AAGCAATGCT	TTTTTATAAT	GCCAACTTTG	TACAAAAAAG	CAGGCTGCAT	GCGAACCAAT
181	TCAGTCGACT	GGATCCGGTA	CCGAATTCGC	TTACTAAAAG	CCAGATAACA	GTATGCGTAT
241	TTGCGCGCTG	ATTTTTCGCG	TATAAGAATA	TATACTGATA	TGTATACCCG	AAGTATGTCA
301	AAAAGAGGTG	TGCTTCTAGA	ATGCACTTTA	AGGTTTACAC	CTATAAAAGA	GAGAGCCGTT
361	ATCGTCTGTT	TGTGGATGTA	CAGAGTGATA	TTATTGACAC	GCCCCGGCGA	CGGATGGTGA
421	TCCCCCTGGC	CAGTGCACGT	CTGCTGTCAG	ATAAAGTCTC	CCGTGAACCT	TACCCGGTGG
481	TGCATATCGG	GGATGAAAGC	TGGCGCATGA	TGACCACCGA	TATGGCCAGT	GTGCCGGTCT
541	CCGTTATCGG	GGAAGAAGTG	GCTGATCTCA	GCCACCGCGA	AAATGACATC	AAAAACGCCA
601	TTAACCTGAT	GTTCTGGGGA	ATATAGAATT	CGCGGCCGCA	CTCGAGATAT	CTAGACCCAG
661	CTTTCCTGTA	CAAAGTTGGC	ATTATAAGAA	AGCATTGCTT	ATCAATTGTG	TGCAACGAAC
721	AGGTCACTAT	CAGTCAAAAT	AAAATCATTA	TTTGCCATCC	AGCTGCAGCT	CTGGCCCGTG
781	TCTCAAAATC	TCTGATGTTA	CATTGCACAA	GATAAAAATA	TATCATCATG	AACAATAAAA
841	CTGTCTGCTT	ACATAAACAG	TAATACAAGG	GGTGTTATGA	GCCATATTCA	ACGGGAAACG
901	TCGAGGCCGC	GATTAAATTC	CAACATGGAT	GCTGATTTAT	ATGGGTATAA	ATGGGCTCGC
961	GATAATGTCT	GGCAATCAGG	TGCGACAATC	TATCGCTTGT	ATGGGAAGCC	CGATGCGCCA
1021	GAGTTGTTTC	TGAAACATGG	CAAAGGTAGC	GTTGCCAATG	ATGTTACAGA	TGAGATGGTC
1081	AGACTAAACT	GGCTGACGGA	ATTTATGCCT	CTTCCGACCA	TCAAGCATTT	TATCCGTACT
1141	CCTGATGATG	CATGGTTACT	CACCACTGCG	ATCCCCGGAA	AAACAGCATT	CCAGGTATTA
1201	GAAGAATATC	CTGATTCAGG	TGAAAATATT	GTTGATGCGC	TGGCAGTGTT	CCTGCGCCGG
1261	TTGCATTCGA	TTCCTGTTTG	TAATTGTCCT	TTTAACAGCG	ATCGCGTATT	TCTGCTCGCT
1321	CAGGCGCAAT	CACGAATGAA	TAACGTTTGG	GTTGATGCGA	GTGATTTTGA	TGACGAGCGT
1381	AATGGCTGGC	CTGTTGAACA	AGTCTGGAAG	GAAATGCATA	AACTTTTGCC	ATTCTCACCG
1441	GATTACAGTC	TCACTCATGG	TGATTTCTCA	CTTGATAACC	TTATTTTGA	CGAGGGGAAA
1501	TTAATAGGTT	GTATTGATGT	TGGACGAGTC	GGAATCGCAG	ACCGATACCA	GGATCTTGCC
1561	ATCCTATGGA	ACTGCCTCGG	TGAGTTTCT	CCTTCATTAC	AGAAACGGCT	TTTTCAAAAA
1621	TATGGTATTG	ATAATCCTGA	TATGAATAAA	TTGCAGTTTC	ATTTGATGCT	CGATGAGTTT
1681	TTCTAATCAG	AATTGGTTAA	TTGGTTGTAA	CATTATTCAG	ATTGGGCCCC	GTTCCACTGA
1741	GCGTCAGACC	CCGTAGAAAA	GATCAAAGGA	TCTTCTTGAG	ATCCTTTTTT	TCTGCGCGTA
1801	ATCTGTGCT	TGCAAAACAA	AAAACCACCG	CTACCAGCGG	TGGTTTGTGT	GCCGGATCAA
1861	GAGCTACCAA	CTCTTTTTTC	GAAGGTAAC	GGCTTCAGCA	GAGCGCAGAT	ACCAAATACT
1921	GTTCTTCTAG	TGTAGCCGTA	GTTAGGCCAC	CACCTCAAGA	ACTCTGTAGC	ACCGCCTACA
1981	TACCTCGCTC	TGCTAATCCT	GTTACCAGTG	GCTGCTGCCA	GTGGCGATAA	GTCGTGTCTT
2041	ACCGGGTTGG	ACTCAAGACG	ATAGTTACCG	GATAAGGCGC	AGCGGTCGGG	CTGAACGGGG
2101	GGTTCGTGCA	CACAGCCCAG	CTTGGAGCGA	ACGACCTACA	CCGAACGTAG	ATACCTACAG
2161	CGTGAGCTAT	GAGAAAGCGC	CACGCTTCCC	GAAGGGAGAA	AGGCGGACAG	GTATCCGGTA
2221	AGCGGCAGGG	TCGGAACAGG	AGAGCGCACG	AGGGAGCTTC	CAGGGGGAAA	CGCCTGGTAT
2281	CTTTATAGTC	CTGTCGGGTT	TCGCCACCTC	TGACTTGAGC	GTCGATTTTT	GTGATGCTCG
2341	TCAGGGGGGC	GGAGCCTATG	GAAAAACGCC	AGCAACGCGG	CCTTTTTTACG	GTTCTTGGCC
2401	TTTTGTCTGG	CTTTTGCTCA	CATGTTCTTT	CCTGCGTTAT	CCCCTGATT	TGTGGATAAC
2461	CGTATTACCG	CTAGCATGGA	TCTCGGGGAC	GTCTAACTAC	TAAGCGAGAG	TAGGGAACGT
2521	CCAGGCATCA	AATAAAACGA	AAGGCTCAGT	CGGAAGACTG	GGCCTTTCGT	TTTATCTGTT
2581	GTTTGTCTGG	GAACGCTCTC	CTGAGTAGGA	CAAATCCGCC	GGGAGCGGAT	TTGAACGTTG
2641	TGAAGCAACG	GCCCCGAGGG	TGGCGGGCAG	GACGCCCGCC	ATAAAGTACC	AGGCATCAAA
2701	CTAAGCAGAA	GGCCATC				

Figure 15B

22/40

Figure 16A: Cloning sites of the ENTRY Vector PENTR7

pENTR7 2738 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
67..166		attL1
342..647		ccdB
676..775		attL2
898..1707		KmR
1812..2385		ori

1	CTGACGGATG	GCCTTTTTCG	GTTTCTACAA	ACTCTTCCTG	TTAGTTAGTT	ACTTAAGCTC
61	GGGCCCCAAA	TAATGATTTT	ATTTTGACTG	ATAGTGACCT	GTTCGTTGCA	ACAAATTGAT
121	AAGCAATGCT	TTTTTATAAT	GCCAACTTTG	TACAAAAAAG	CAGGCTTTGA	AAACCTGTAT
181	TTTCAAGGAA	CCGTTTCATG	CATCGTCGAC	TGGATCCGGT	ACCGAATTCG	CTTACTAAAA
241	GCCAGATAAC	AGTATGCGTA	TTTGCGCGCT	GATTTTTCG	GTATAAGAAT	ATATACTGAT
301	ATGTATACCC	GAAGTATGTC	AAAAAGAGGT	GTGCTTCTAG	AATGCAGTTT	AAGGTTTACA
361	CCTATAAAAG	AGAGAGCCGT	TATCGTCTGT	TTGTGGATGT	ACAGAGTGAT	ATTATTGACA
421	CGCCCGGGCG	ACGGATAGTG	ATCCCCCTGG	CCAGTGCACG	TCTGCTGTCA	GATAAAGTCT
481	CCCGTGAAC	TTACCCGGTG	GTGCATATCG	GGGATGAAAG	CTGGCGCATG	ATGACCACCG
541	ATATGGCCAG	TGTGCCGGTC	TCCGTTATCG	GGGAAGAAGT	GGCTGATCTC	AGCCACCGCG
601	AAAATGACAT	CAAAAACGCC	ATTAACCTGA	TGTTCTGGGG	AATATAGAAT	TCGCGGCCGC
661	ACTCGAGATA	TCTAGACCCA	GCTTCTTGT	ACAAAGTTGG	CATTATAAGA	AAGCATTGCT
721	TATCAATTG	TTGCAACGAA	CAGGTCAC	TCAGTCAAAA	TAAAATCATT	ATTTGCCATC
781	CAGCTGCAGC	TCTGGCCCGT	GTCTCAAAAT	CTCTGATGTT	ACATTGCACA	AGATAAAAAT
841	ATATCATCAT	GAACAATAAA	ACTGTCTGCT	TACATAAACA	GTAATACAAG	GGGTGTTATG
901	AGCCATATTC	AACGGGAAAC	GTCGAGGCCG	CGATTAAATT	CCAACATGGA	TGCTGATTTA
961	TATGGGTATA	AATGGGCTCG	CGATAATGTC	GGGCAATCAG	GTGCGACAAT	CTATCGCTTG
1021	TATGGGAAGC	CCGATGCGCC	AGAGTTGTTT	CTGAAACATG	GCAAAGGTAG	CGTTGCCAAT
1081	GATGTTACAG	ATGAGATGGT	CAGACTAAAC	TGGCTGACGG	AATTTATGCC	TCTTCCGACC
1141	ATCAAGCATT	TTATCCGTAC	TCCTGATGAT	GCATGGTTAC	TCACCACTGC	GATCCCCGGA
1201	AAAACAGCAT	TCCAGGTATT	AGAAGAATAT	CCTGATTCAG	GTGAAAATAT	TGTTGATGCG
1261	CTGGCAGTGT	TCCTGCGCCG	GTTGCATTTC	ATTCTGTGTT	GTAATTGTCC	TTTTAACAGC
1321	GATCGCGTAT	TTCGTCTCGC	TCAGGCGCAA	TCACGAATGA	ATAACGGTTT	GGTTGATGCG
1381	AGTGATTTTG	ATGACGAGCG	TAATGGCTGG	CCTGTTGAAC	AAGTCTGGAA	AGAAATGCAT
1441	AACTTTTTCG	CATTCTCACC	GGATTTCAGT	GTCACATCAT	GTGATTTCTC	ACTTGATAAC
1501	CTTATTTTTC	ACGAGGGGAA	ATTAATAGGT	TGTATTGATG	TTGGACGAGT	CGGAATCGCA
1561	GACCGATACC	AGGATCTTGC	CATCCTATGG	AACTGCCTCG	GTGAGTTTTT	TCCTTCATTA
1621	CAGAAACGCG	TTTTTCAAAA	ATATGGTATT	GATAATCCTG	ATATGAATAA	ATTGCAGTTT
1681	CATTTGATGC	TCGATGAGTT	TTTCTAATCA	GAATTGGTTA	ATTGGTTGTA	ACATTATTCA
1741	GATTGGGCCC	CGTTCCACTG	AGCGTCAGAC	CCCGTAGAAA	AGATCAAAGG	ATCTTCTTGA
1801	GATCCTTTT	TTCTGCGCGT	AATCTGCTGC	TTGCAAACAA	AAAAACCACC	GCTACCAGCG
1861	GTGGTTTGTT	TGCCGGATCA	AGAGCTACCA	ACTCTTTTTC	CGAAGGTAAC	TGGCTTCAGC
1921	AGAGCGCAGA	TACCAAATAC	TGTTCTTCTA	GTGTAGCCGT	AGTTAGGCCA	CCACTTCAAG
1981	AACTCTGTAG	CACCGCCTAC	ATACCTCGCT	CTGCTAATCC	TGTTACCAGT	GGCTGCTGCC
2041	AGTGGCGATA	AGTCGTGTCT	TACCGGGTTG	GACTCAAGAC	GATAGTTACC	GGATAAGGCG
2101	CAGCGGTCGG	GCTGAACGGG	GGGTTCTGTC	ACACAGCCCA	GCTTGGAGCG	AACGACCTAC
2161	ACCGAACTGA	GATACCTACA	GCGTGAGCTA	TGAGAAAGCG	CCACGCTTCC	CGAAGGGAGA
2221	AAGGCGGACA	GGTATCCGGT	AAGCGGCAGG	GTCGGAACAG	GAGAGCGCAC	GAGGGAGCTT
2281	CCAGGGGGAA	ACGCCTGGTA	TCTTTATAGT	CCTGTCCGGT	TTCGCCACCT	CTGACTTGAG
2341	CGTCGATTTT	TGTGATGCTC	GTCAGGGGGG	CGGAGCCTAT	GGAAAAACGC	CAGCAACGCG
2401	GCCTTTTAC	GGTTCTTGGC	CTTTTGCTGG	CCTTTGCTC	ACATGTTCTT	TCCTGCGTTA
2461	TCCCCTGATT	CTGTGGATAA	CCGTATTACC	GCTAGCATGG	ATCTCGGGGA	CGTCTAACTA
2521	CTAAGCGAGA	GTAGGGAAC	GCCAGGCATC	AAATAAAACG	AAAGGCTCAG	TCGGAAGACT
2581	GGGCCTTTTC	TTTTATCTGT	TGTTTGTCGG	TGAACGCTCT	CCTGAGTAGG	ACAAATCCGC
2641	CGGGAGCGGA	TTTGAACGTT	GTAAGCAAC	GGCCCGGAGG	GTGGCGGGCA	GGACGCCCGC
2701	CATAAACTGC	CAGGCATCAA	ACTAAGCAGA	AGGCCATC		

Figure 16B

24/240

Figure 17A: Cloning Sites of the *ENTRY* Vector: pENTRY8

Int attC1

ctt tac aaa aaa gca ggc ttt	gaa aac ctg tat ttt caa gya
gag atg gtc ttt cgt ccg aaa	ctt ttg gac ata aaa gtt cct

Leu Tyr Lys Lys Ala Gly Phe Glu Asn Leu Tyr Phe Gln Gly

TEV Protease

NotI AatII SalI BamHI KpnI EcoRI

act atg gac cta gtc gac tgg atc cgg tac cda att cgc ---
tgg tac ctg gat cag ctg acc tag gct atg gct taa gcg ---

Thr Met Asp Leu Val Asp Trp Ile Arg Tyr Arg Ile

EcoRI NotI XhoI EcoRV XbaI attC

Death --- aga att cgc ggc cgc act cga gat atc tag acc cag
--- tct taa gcg cgc ggc tga gct cta tag atc tgg gtc

Int

ctt tct gtc aca aag ---
gaa aga aca tgt ctc ---

25/240

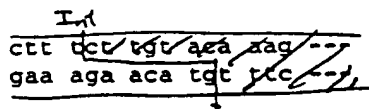
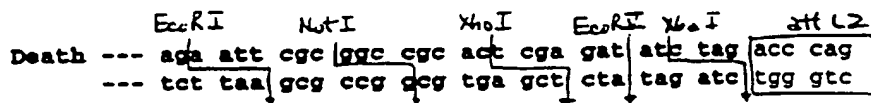
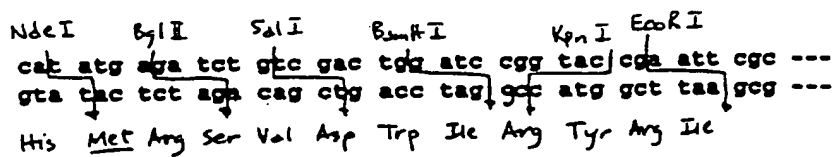
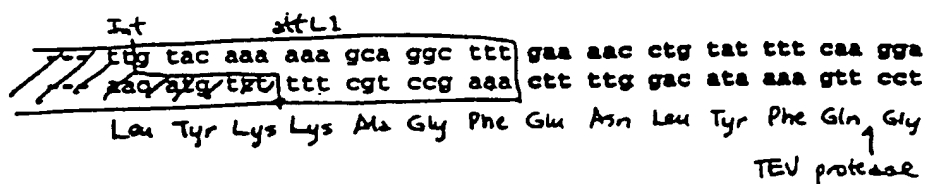
pENTR8 2735 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
67..166		attL1
339..644		ccdB
673..772		attL2
895..1704		KmR
1809..2382		ori
1	CTGACGGATG GCCTTTTTGC GTTCTACAA ACTCTTCCTG TTAGTTAGTT ACTTAAGCTC	
61	GGGCCCCAAA TAATGATTTT ATTTGACTG ATAGTGACCT GTTCGTTGCA ACAAATTGAT	
121	AAGCAATGCT TTTTATAAT GCCAACTTTG TACAAAAAAG CAGGCTTTGA AAACCTGTAT	
181	TTTCAAGGAA CCATGGACCT AGTCGACTGG ATCCGGTACC GAATTCGCTT ACTAAAAGCC	
241	AGATAACAGT ATGCGTATTT GCGCGCTGAT TTTTGCGGTA TAAGAATATA TACTGATATG	
301	TATACCCGAA GTATGTCAA AAGAGGTGTG CTCTAGAAT GCAGTTTAAG GTTTACACCT	
361	ATAAAAGAGA GAGCCGTTAT CGTCTGTTTG TGGATGTACA GAGTGATATT ATTGACACGC	
421	CCGGGCGACG GATAGTGATC CCCCTGGCCA GTGCACGTCT GCTGTCAGAT AAAGTCTCCC	
481	GTGAACCTTA CCCGGTGGTG CATATCGGGG ATGAAAGCTG GCGCATGATG ACCACCGATA	
541	TGGCCAGTGT GCCGGTCTCC GTTATCGGGG AAGAAGTGGC TGATCTCAGC CACCGCGAAA	
601	ATGACATCAA AAACGCCATT AACCTGATGT TCTGGGGAAT ATAGAATTCTG CGGCCGCACT	
661	CGAGATATCT AGACCCAGCT TTCTTGTAACA AAGTTGGCAT TATAAGAAAG CATTGCTTAT	
721	CAATTTGTTG CAACGAACAG GCACATATCA GTCAAAATAA AATCATTATT TGCCATCCAG	
781	CTGCAGCTCT GGCCCGTGTC TCAAAATCTC TGATGTTACA TTGCACAAGA TAAAAATATA	
841	TCATCATGAA CAATAAACT GTCTGCTTAC ATAAACAGTA ATACAAGGGG TGTATGAGC	
901	CATATTCAAC GGGAAACGTC GAGGCCGCGA TTAAATTCCA ACATGGATGC TGATTATAT	
961	GGGTATAAAT GGGCTCGCGA TAATGTCGGG CAATCAGGTG CGACAATCTA TCGCTTGTAT	
1021	GGGAAGCCCG ATGCGCCAGA GTTGTTTCTG AAACATGGCA AAGGTAGCGT TGCCAATGAT	
1081	GTTACAGATG AGATGGTCAG ACTAACTGG CTGACGGAAT TTATGCTCTT TCCGACCATC	
1141	AAGCATTTTA TCCGTACTCC TGATGATGCA TGGTTACTCA CCACTGCGAT CCCCAGAAAA	
1201	ACAGCATTCC AGGTATTAGA AGAATATCCT GATTCAGGTG AAAATATTGT TGATGCGCTG	
1261	GCAAGTCCCG TGCGCCGGTT GCATTGATT CCTGTTTGTA ATTGTCCTTT TAACAGCGAT	
1321	CGCGTATTTC GTCTCGCTCA GCGCAATCA CGAATGAATA ACGGTTTGGT TGATGCGAGT	
1381	GATTTTGATG ACGAGCGTAA TGGCTGCGCT GTTGAACAAG TCTGGAAAGA AATGCATAAA	
1441	CTTTTGCCAT TCTCACCGGA TTCAGTCGTC ACTCATGGTG ATTTCTCACT TGATAACCTT	
1501	ATTTTGTACG AGGGGAAATT AATAGGTTGT ATTGATGTTG GACGAGTCGG AATCGCAGAC	
1561	CGATACCAGG ATCTTGCCAT CCTATGGAAC TGCCTCGGTG AGTTTTCTCC TTCATTACAG	
1621	AAACGGCTTT TTCAAAAATA TGGTATTGAT AATCCTGATA TGAATAAATT GCAGTTTCAT	
1681	TTGATGCTCG ATGAGTTTTT CTAATCAGAA TTGGTTAATT GGTGTAACA TTATTACAGT	
1741	TGGGCCCCGT TCCACTGAGC GTCAGACCCC GTAGAAAAGA TCAAAGGATC TTCTTGAGAT	
1801	CCTTTTTTTC TGCAGCTAAT CTGCTGCTTG CAAACAAAAA AACCACCGCT ACCAGCGGTG	
1861	GTTTGTTTGC CGGATCAAGA GCTACCAACT CTTTTTCCGA AGGTAACCTGG CTTACAGAGA	
1921	GCGCAGATAC CAAATACTGT TCTTCTAGTG TAGCCGTAGT TAGGCCACCA CTTCAAGAAC	
1981	TCTGTAGCAC CGCCTACATA CCTCGCTCTG CTAATCCTGT TACCAGTGGC TGCTGCCAGT	
2041	GGCGATAAGT CGTGTCTTAC CGGGTTGGAC TCAAGACGAT AGTTACCGGA TAAGGCGCAG	
2101	CGGTCGGGCT GAACGGGGG TTCGTGCACA CAGCCCAGCT TGGAGCGAAC GACCTACACC	
2161	GAAGTGAAGT ACCTACAGCG TGAGCTATGA GAAAGCGCCA CGCTTCCCGA AGGGAGAAAAG	
2221	GCGGACAGGT ATCCGGTAAG CCGCAGGGTC GGAACAGGAG AGCGCACGAG GGAGCTTCCA	
2281	GGGGGAAACG CCTGGTATCT TTATAGTCCT GTCGGGTTTC GCCACCTCTG ACTTGAGCGT	
2341	CGATTTTTGT GATGCTCGTC AGGGGGGCGG AGCCTATGGA AAAACGCCAG CAACGCGGCC	
2401	TTTTTACGGT TCCTGGCCTT TTGCTGGCCT TTTGCTCACA TGTTCTTTCC TGCGTTATCC	
2461	CCTGATTCTG TGGATAACCG TATTACCGCT AGCATGGATC TCGGGGACGT CTAACACTA	
2521	AGCGAGAGTA GGGAACTGCC AGGCATCAAA TAAACGAAA GGCTCAGTCG GAAGACTGGG	
2581	CCTTTCGTTT TATCTGTTGT TTGTCGGTGA ACGCTCTCCT GAGTAGGACA AATCCGCCCG	
2641	GAGCGGATTT GAACGTTGTG AAGCAACGCG CCGGAGGGTG GCGGGCAGGA CGCCGCCCAT	
2701	AAACTGCCAG GCATCAAAC AAGCAGAAGG CCATC	

FIGURE 17B

26/240

Figure 18A: Cloning sites of the ENTRY Vector pENTRY



27/240

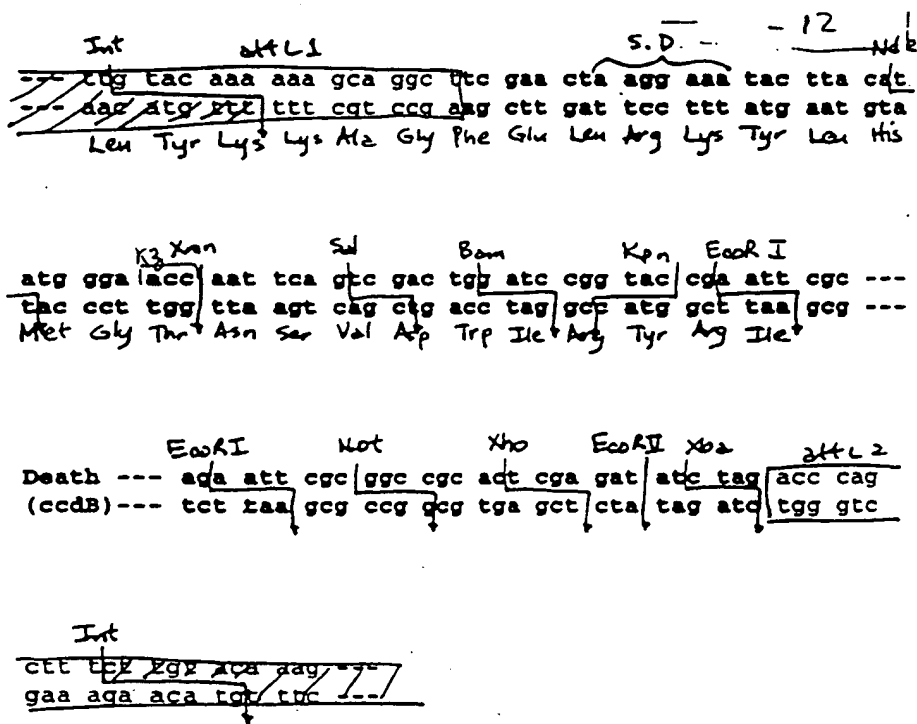
pENTR9 2735 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
67..166		attL1
339..644		ccdB
673..772		attL2
895..1704		KmR
1809..2382		ori
1	CTGACGGATG GCCTTTTTCG GTTTCTACAA ACTCTTCTCG TTAGTTAGTT ACTTAAGCTC	
61	GGGCCCCAAA TAATGATTTT ATTTTGACTG ATAGTGACCT GTTCGTTGCA ACAAATTGAT	
121	AAGCAATGCT TTTTATAAAT GCCAACTTTG TACAAAAAAG CAGGCTTTGA AAACCTGTAT	
181	TTTCAAGGAC ATATGAGATC TGTGCGACTGG ATCCGGTACC GAATTCGCTT ACTAAAAGCC	
241	AGATAACAGT ATGCGTATTT GCGCGCTGAT TTTTGCGGTA TAAGAATATA TACTGATATG	
301	TATACCCGAA GTATGTCAAA AAGAGGTGTG CTTCTAGAAT GCAGTTTAAG GTTTACACCT	
361	ATAAAAGAGA GAGCCGTTAT CGTCTGTTTG TGGATGTACA GAGTGATATT ATTGACACGC	
421	CCGGGCGACG GATAGTGATC CCCCTGGCCA GTGCACGTCT GCTGTCAGAT AAAGTCTCCC	
481	GTGAACTTTA CCCGGTGGTG CATATCGGGG ATGAAAGCTG GCGCATGATG ACCACCGATA	
541	TGGCCAGTGT GCCGGTCTCC GTTATCGGGG AAGAAGTGGC TGATCTCAGC CACCGCGAAA	
601	ATGACATCAA AAACGCCATT AACCTGATGT TCTGGGGAAT ATAGAATTCT CGGCCGCACT	
661	CGAGATATCT AGACCCAGCT TTCTTGATACA AAGTTGGCAT TATAAGAAAG CATTGCTTAT	
721	CAATTTGTTG CAACGAACAG GTCACATCA GTCAAAATAA AATCATTATT TGCCATCCAG	
781	CTGCAGCTCT GGCCCGTGTG TCAAAATCTC TGATGTTACA TTGCACAAGA TAAAAATATA	
841	TCATCATGAA CAATAAAACT GTCTGCTTAC ATAAACAGTA ATACAAGGGG TGTTATGAGC	
901	CATATTCAAC GGGAAACGTC GAGGCCGCGA TTAAATTCCA ACATGGATGC TGATTTATAT	
961	GGGTATAAAT GGGCTCGCGA TAATGTCGGG CAATCAGGTG CGACAATCTA TCGCTTGTAT	
1021	GGGAAGCCCG ATGCGCCAGA GTTGTTCCTG AAACATGGCA AAGGTAGCGT TGCCAATGAT	
1081	GTTACAGATG AGATGGTCAG ACTAACTGG CTGACGGAAT TTATGCCTCT TCCGACCATC	
1141	AAGCATTTTA TCCGTACTCC TGATGATGCA TGGTTACTCA CCACTGCGAT CCCCAGAAAA	
1201	ACAGCATTCC AGGTATTAGA AGAATATCCT GATTCAGGTG AAAATATTGT TGATGCGCTG	
1261	GCAGTGTCCT TGCGCCGGTT GCATTCGATT CCTGTTTGTA ATTGTCCTTT TAACAGCGAT	
1321	CGCGTATTTT GTCTCGCTCA GGCAGCAATCA CGAATGAATA ACGGTTTGGT TGATGCGAGT	
1381	GATTTTGATG ACGAGCGTAA TGGCTGGCCT GTTGAACAAG TCTGGAAAGA AATGCATAAA	
1441	CTTTTGCCAT TCTCACCAGA TTCAGTCGTC ACTCATGGTG ATTTCTCACT TGATAACCTT	
1501	ATTTTGTACG AGGGGAAATT AATAGGTTGT ATTGATGTTG GACGAGTCGG AATCGCAGAC	
1561	CGATACCAGG ATCTTGCCAT CCTATGGAAC TGCCTCGGTG AGTTTCTCTC TTCATTACAG	
1621	AAACGCGCTT TTCAAAAATA TGGTATTGAT AATCCTGATA TGAATAAATT GCAGTTTCAT	
1681	TTGATGCTCG ATGAGTTTTC CTAATCAGAA TTGGTTAATT GGTGTAACA TTATTAGAT	
1741	TGGGCCCGGT TCCACTGAGC GTCAGACCCC GTAGAAAAGA TCAAAGGATC TTCTTGAGAT	
1801	CCTTTTTCCT TCGCGTAAT CTGCTGCTTG CAAACAAAAA AACCACCGCT ACCAGCGGTG	
1861	GTTTGTTCCT CGGATCAAGA GCTACCAACT CTTTTCCGA AGGTAAGTGG CTTCAGCAGA	
1921	GCGCAGATAC CAAATACTGT TCTTCTAGTG TAGCCGTAGT TAGGCCACCA CTTCAGAAC	
1981	TCTGTAGCAC CGCCTACATA CCTCGCTCTG CTAATCCTGT TACCAGTGGC TGCTGCCAGT	
2041	GGCGATAAGT CGTGTCTTAC CGGGTTGGAC TCAAGACGAT AGTTACCGGA TAAGGCGCAG	
2101	CGGTCGGGCT GAACGGGGGG TTCGTGCACA CAGCCCAGCT TGGAGCGAAC GACCTACACC	
2161	GAAGTACGAT ACCTACAGCG TGAGCTATGA GAAAGCGCCA CGCTTCCCGA AGGGAGAAAG	
2221	GCGGACAGGT ATCCGGTAAG CGGCAGGGTC GGAACAGGAG AGCGCACGAG GGAGCTTCCA	
2281	GGGGGAAACG CCTGGTATCT TTATAGTCCT GTCGGGTTTC GCCACCTCTG ACTTGAGCGT	
2341	CGATTTTGTG GATGCTCGTC AGGGGGGCGG AGCCTATGGA AAAACGCCAG CAACGCGGCC	
2401	TTTTTACGGT TCCTGGCCTT TTGCTGGCCT TTTGCTCACA TGTTCTTTCC TGCGTTATCC	
2461	CCTGATTCTG TGATAACCG TATTACCGCT AGCATGGATC TCGGGGACGT CTAATACTA	
2521	AGCGAGAGTA GGGAACTGCC AGGCATCAAA TAAAACGAAA GGCTCAGTCG GAAGACTGGG	
2581	CCTTTCGTTT TATCTGTTGT TTGTCGGTGA ACGCTCTCCT GAGTAGGACA AATCCGCCGG	
2641	GAGCGGATTT GAACGTTGTG AAGCAACGGC CCGGAGGGTG GCGGGCAGGA CGCCGCCCAT	
2701	AAACTGCCAG GCATCAAAC AAGCAGAAGG CCATC	

Figure 18B

28/240

Figure 19A: Cloning sites of the ENTRY Vector pENTRY10



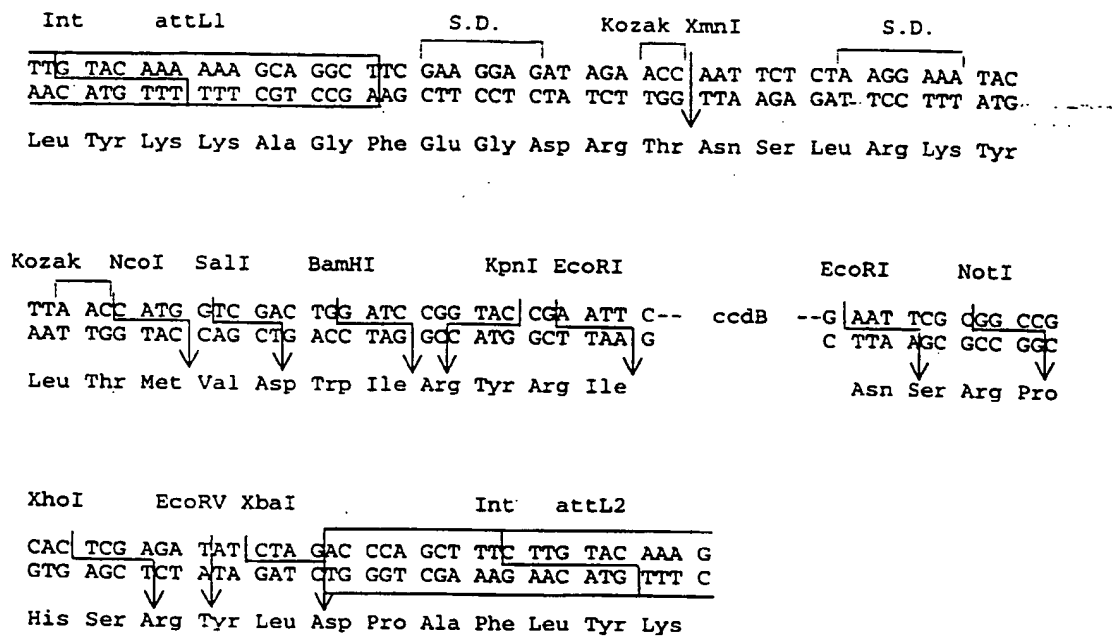
29/240

pENTR10 2738 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
67..166		attL1
342..647		ccdB
676..775		attL2
898..1707		KmR
1812..2385		ori
1	CTGACGGATG GCCTTTTTCG GTTCTACAA	ACTCTTCTCG TTAGTTAGTT ACTTAAGCTC
61	GGGCCCCAAA TAATGATTTT ATTTTGACTG	ATAGTGACCT GTTCGTTGCA ACAAATTGAT
121	AAGCAATGCT TTTTATAAT GCCAATTTG	TACAAAAAAG CAGGCTTCGA ACTAAGGAAA
181	TACTTACATA TGGGAACCAA TTCAGTCGAC	TGGATCCGGT ACCGAATTCTG CTTACTAAAA
241	GCCAGATAAC AGTATGCGTA TTTGCGCGCT	GATTTTTGCG GTATAAGAAT ATATACTGAT
301	ATGTATACCC GAAGTATGTC AAAAAGAGGT	GTGCTTCTAG AATGCAGTTT AAGGTTTACA
361	CCTATAAAAAG AGAGAGCCGT TATCGTCTGT	TTGTGGATGT ACAGAGTGAT ATTATTGACA
421	CGCCCGGGCG ACGGATGGTG ATCCCCCTGG	CCAGTGCACG TCTGCTGTCA GATAAAGTCT
481	CCCGTGAAC TTTACCCGGTG GTGCATATCG	GGGATGAAAG CTGGCGCATG ATGACCACCG
541	ATATGGCCAG TGTGCCGGTC TCCGTTATCG	GGGAAGAAGT GGCTGATCTC AGCCACCCCG
601	AAAATGACAT CAAAAACGCC ATTAACCTGA	TGTTCTGGGG AATATAGAAT TCGCGGCCGC
661	ACTCGAGATA TCTAGACCCA GCTTTCCTGT	ACAAAGTTGG CATTATAAGA AAGCATTGCT
721	TATCAATTG TTGCAACGAA CAGGTCACCTA	TCAGTCAAAA TAAAATCATT ATTTGCCATC
781	CAGCTGCAGC TCTGGCCCGT GTCTCAAAAT	CTCTGATGTT ACATTGCACA AGATAAAAAT
841	ATATCATCAT GAACAATAAA ACTGTCTGCT	TACATAAACA GTAATACAAG GGGTGTTATG
901	AGCCATATTC AACGGGAAAC GTCGAGGCCG	CGATTAAATT CCAACATGGA TGCTGATTTA
961	TATGGGTATA AATGGGCTCG CGATAATGTC	GGGCAATCAG GTGCGACAAT CTATCGCTTG
1021	TATGGGAAGC CCGATGCGCC AGAGTTGTTT	CTGAAACATG GCAAAGGTAG CGTTGCCAAT
1081	GATGTTACAG ATGAGATGGT CAGACTAAAC	TGGCTGACGG AATTTATGCC TCTCCGACC
1141	ATCAAGCATT TTATCCGTAC TCCTGATGAT	GCATGGTTAC TCACCACTGC GATCCCCGGA
1201	AAAACAGCAT TCCAGGTATT AGAAGAATAT	CCTGATTACG GTGAAAATAT TGTGATGCG
1261	CTGGCAGTGT TCCTGCGCCG GTTGCAATCG	ATTCTGTTT GTAATTGTCC TTTTAACAGC
1321	GATCGCGTAT TTCGTCTCGC TCAGGCGCAA	TCACGAATGA ATAACGGTTT GGTGATGCG
1381	AGTGATTTTG ATGACGAGCG TAATGGCTGG	CCTGTGAAC AAGTCTGGA AGAAATGCAT
1441	AACTTTTTCG CATTCTCACC GGATTCAGTC	GTCACTCATG GTGATTTCTC ACTTGATAAC
1501	CTTATTTTTCG ACGAGGGGAA ATTAATAGGT	TGTATTGATG TTGGACGAGT CGGAATCGCA
1561	GACCGATAAC AGGATCTTGC CATCCTATGG	AAGTGCCTCG GTGAGTTTTC TCCTTCATTA
1621	CAGAAACGGC TTTTTCAAAA ATATGGTATT	GATAATCCTG ATATGAATAA ATTGCAGTTT
1681	CATTTGATGC TCGATGAGTT TTTCTAATCA	GAATTGGTTA ATTGGTTGTA ACATTATTCA
1741	GATTGGGCCC CGTTCCACTG AGCGTCAGAC	CCCGTAGAAA AGATCAAAGG ATCTTCTTGA
1801	GATCCTTTT TTTCTGCGCT AATCTGCTGC	TTGCAAACAA AAAAACCACC GCTACCAGCG
1861	GTGGTTTGTT TGCCGGATCA AGAGCTACCA	ACTCTTTTTC CGAAGGTAAC TGGCTTCAGC
1921	AGAGCGCAGA TACCAAATAC TGTTCTTCTA	GTGTAGCCGT AGTTAGGCCA CCACCTCAAG
1981	AACCTCTGTAG CACCGCCTAC ATACCTCGCT	CTGCTAATCC TGTACCAGT GGCTGCTGCC
2041	AGTGGCGATA AGTCGTGTCT TACCGGTTG	GACTCAAGAC GATAGTTACC GGATAAGGCG
2101	CAGCGGTCGG GCTGAACGGG GGGTTCGTGC	ACACAGCCCA GCTTGGAGCG AACGACCTAC
2161	ACCGAACTGA GATACCTACA GCGTGAGCTA	TGAGAAAGCG CCACGCTTCC CGAAGGGAGA
2221	AAGGCGGACA GGTATCCGGT AAGCGGCAGG	GTCGGAACAG GAGAGCGCAC GAGGGAGCTT
2281	CCAGGGGGAA ACGCCTGGTA TCTTTATAGT	CCTGTCCGGT TTCGCCACCT CTGACTTGAG
2341	CGTCGATTTT TGTGATGCTC GTCAGGGGGG	CGGAGCCTAT GGAAAAACGC CCAACGCG
2401	GCCTTTTAC GGTTCCTGGC CTTTGTCTGG	CCTTTGCTC ACATGTTCTT TCCTCGTTA
2461	TCCCCTGATT CTGTGGATAA CCGTATTACC	GCTAGCATGG ATCTCGGGGA CGTCTAATA
2521	CTAAGCGAGA GTAGGGAAC GCCAGGCATC	GAATAAAACG AAAGGCTCAG TCGGAAGACT
2581	GGGCCTTTTCG TTTTATCTGT TGTGTGTCGG	TGAACGCTCT CCTGAGTAGG ACAAATCCGC
2641	CGGGAGCGGA TTTGAACGTT GTGAAGCAAC	GGCCCGGAGG GTGGCGGGCA GGACGCCCGC
2701	CATAAACTGC CAGGCATCAA ACTAAGCAGA	AGGCCATC

FIGURE 19B

30/240

Figure 20A: Cloning Sites of the Entry Vector pENTR11

pENTR11 2744 bp (rotated to position 2578)

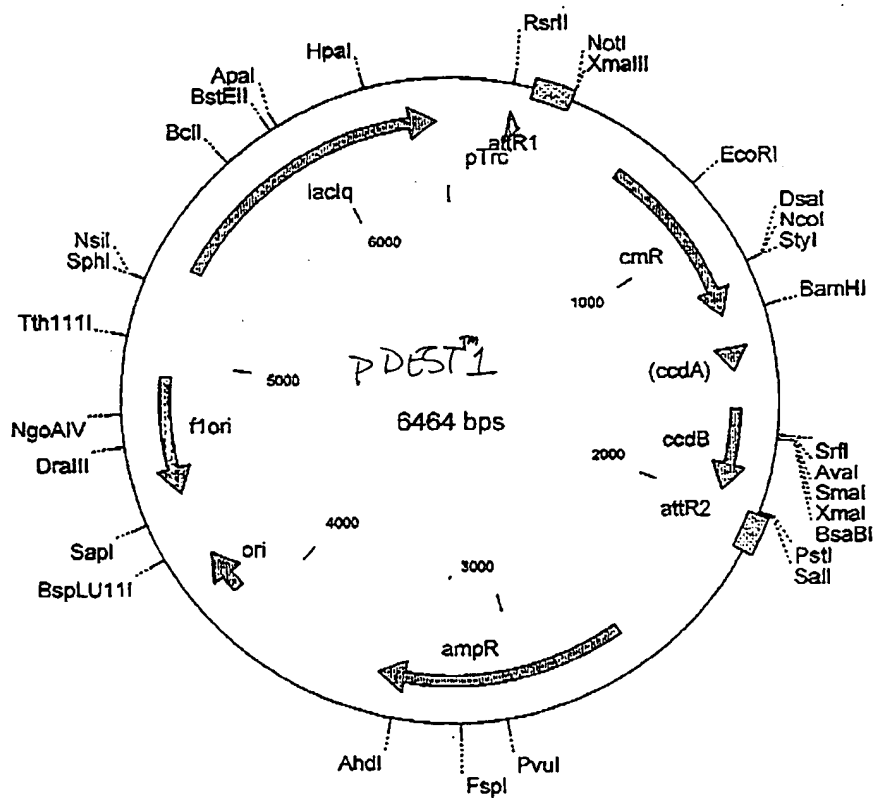
<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
67..166		attL1
348..653		ccdB
683..781		attL2
904..1713		KmR
1818..2391		ori

1	CTGACGGATG	GCCTTTTTC	GTTTCTACAA	ACTCTTCCTG	TTAGTTAGTT	ACTTAAGCTC
61	GGGCCCCAAA	TAATGATTTT	ATTTTGACTG	ATAGTGACCT	GTTCGTTGCA	ACAAATTGAT
121	AAGCAATGCT	TTTTTATAAT	GCCAACTTTG	TACAAAAAAG	CAGGCTTCGA	AGGAGATAGA
181	ACCAATTCTC	TAAGGAAATA	CTTAACCATG	GTCGACTGGA	TCCGGTACCG	AATTCGCTTA
241	CTAAAAGCCA	GATAACAGTA	TGCGTATTTG	CGCGCTGATT	TTTGCGGTAT	AAGAATATAT
301	ACTGATATGT	ATACCCGAAG	TATGTCAAAA	AGAGGTGTGC	TTCTAGAATG	CAGTTTAAGG
361	TTTACACCTA	TAAAAGAGAG	AGCCGTTATC	GTCTGTTTGT	GGATGTACAG	AGTGATATTA
421	TTGACACGCC	CGGGCGACGG	ATAGTGATCC	CCCTGGCCAG	TGCACGCTCG	CTGTCTAGATA
481	AAGTCTCCTG	TGAACCTTAC	CCGGTGGTGC	ATATCGGGGA	TGAAAGCTGG	CGCATGTATGA
541	CCACCGATAT	GGCCAGTGTG	CCGGTCTCCG	TTATCGGGGA	AGAAGTGGCT	GATCTCAGCC
601	ACCGCGAAAA	TGACATCAAA	AACGCCATTA	ACCTGATGTT	CTGGGGAATA	TAGAATTCCG
661	GGCGCACTC	GAGATATCTA	GACCCAGCTT	TCTTGATCAA	AGTTGGCATT	ATAAGAAAGC
721	ATTGCTTATC	AATTTGTTGC	AACGAACAGG	TCACTATCAG	TCAAAATAAA	ATCATTATTT
781	GCCATCCAGC	TGCAGCTCTG	GCCCGTGTCT	CAAAATCTCT	GATGTTACAT	TGCACAAGAT
841	AAAAATATAT	CATCATGAAC	AATAAACTG	TCTGCTTACA	TAAACAGTAA	TACAAGGGGT
901	GTTATGAGCC	ATATTCAACG	GGAAACGTCG	AGGCCGCGAT	TAAATTCCAA	CATGGATGCT
961	GATTTATATG	GGTATAAATG	GGCTCGCGAT	AATGTCGGGC	AATCAGGTGC	GACAATCTAT
1021	CGCTTGTATG	GGAAGCCCGA	TGCGCCAGAG	TTGTTTCTGA	AACATGGCAA	AGGTAGCGTT
1081	GCCAATGATG	TTACAGATGA	GATGGTCAGA	CTAAACTGGC	TGACGGAATT	TATGCCTCTT
1141	CCGACCATCA	AGCATTTTAT	CCGTACTCCT	GATGATGCAT	GGTACTCAC	CACTGCGATC
1201	CCCGGAAAAA	CAGCATTCCA	GGTATTAGAA	GAATATCCTG	ATTGAGGTGA	AAATATTGTT
1261	GATGCGCTGG	CAGTGTTCCT	GCGCCGGTTG	CATTCGATTG	CTGTTTGTA	TTGTCCTTTT
1321	AACAGCGATC	GCGTATTTTC	TCTCGCTCAG	GCGCAATCAC	GAATGAATAA	CGGTTTGGTT
1381	GATGCGAGTG	ATTTTGATGA	CGAGCGTAAT	GGCTGGCCTG	TTGAACAAGT	CTGGAAAGAA
1441	ATGCATAAAC	TTTTTGCCATT	CTCACCAGGAT	TCAGTCGTCA	CTCATGGTGA	TTTCTCACTT
1501	GATAACCTTA	TTTTTGACGA	GGGGAAATTA	ATAGGTTGTA	TTGATGTTGG	ACGAGTCGGA
1561	ATCGCAGACC	GATACCAGGA	TCTTGCCATC	CTATGGAACT	GCCTCGGTGA	GTTTTCTCCT
1621	TCATTACAGA	AACGGCTTTT	TCAAAAATAT	GGTATTGATA	ATCCTGATAT	GAATAAATTG
1681	CAGTTTCATT	TGATGCTCGA	TGAGTTTTTC	TAATCAGAAT	TGGTTAATTG	GTTGTAACAT
1741	TATTCAGATT	GGGCCCGGTT	CCAATGAGCG	TCAGACCCCG	TAGAAAAGAT	CAAAGGATCT
1801	TCTTGAGATC	CTTTTTTTCT	GCGCGTAATC	TGCTGCTTGC	AAACAAAAAA	ACCACCGCTA
1861	CCAGCGGTGG	TTTGTTTGCC	GGATCAAGAG	CTACCAACTC	TTTTTCCGAA	GGTAACTGGC
1921	TTCAGCAGAG	CGCAGATACC	AAATACTGTT	CTTCTAGTGT	AGCCGTAGTT	AGGCCACCAC
1981	TTCAAGAACT	CTGTAGCACC	GCCTACATAC	CTCGCTCTGC	TAATCCTGTT	ACCAGTGGCT
2041	GCTGCCAGTG	GCGATAAGTC	GTGTCTTACC	GGGTGGGACT	CAAGACGATA	GTTACCGGAT
2101	AAGGCGCAGC	GGTCGGGCTG	AACGGGGGGT	TCGTGCACAC	AGCCCAGCTT	GGAGCGAAGC
2161	ACCTACACCG	AACTGAGATA	CCTACAGCGT	GAGCTATGAG	AAAGCGCCAC	GCTTCCCGAA
2221	GGGAGAAAGG	CGGACAGGTA	TCCGGTAAGC	GGCAGGGTCG	GAACAGGAGA	GCGCACGAGG
2281	GAGCTTCCAG	GGGGAAACGC	CTGGTATCTT	TATAGTCCTG	TCGGGTTTCG	CCACCTCTGA
2341	CTTGAGCGTC	GATTTTGTGT	ATGCTCGTCA	GGGGGGCGGA	GCCTATGGAA	AAACGCCAGC
2401	AACGCGGCCT	TTTTACGGTT	CCTGGCCTTT	TGCTGGCCTT	TTGCTCACAT	GTTCTTTCTT
2461	GCGTTATCCC	CTGATTCTGT	GGATAACCGT	ATTACCGCTA	GCATGGATCT	CGGGGACGTC
2521	TAGACTACTAA	GCGAGAGTAG	GGAAGTGGCA	GGCATCAAAT	AAAACGAAAG	GCTCAGTCGG
2581	AAGACTGGGC	CTTTCGTTTT	ATCTGTTGTT	TGTCGGTGAA	CGCTCTCCTG	AGTAGGACAA
2641	ATCCGCCGGG	AGCGGATTTG	AACGTTGTGA	AGCAACGGCC	CGGAGGGTGG	CGGGCAGGAC
2701	GCCCCCCATA	AACTGCCAGG	CATCAAACCTA	AGCAGAAGGC	CATC	

FIGURE 20B

Figure 2: pDEST1 Native Protein Expression in E. coli

1 atgagctg ⁻³⁵ gacattaat ^{Tre promoter} catccggctc ⁻¹⁰ gataatgtg ^{RNA} tggattgtg agcggataac
 tactcgacaa ctgttaatta gtaggccgag catattacac accttaacac tcgctattg
 61 aatttcacac aggaacacaga caggtatagg atcacaagtt ^{attR1} ~~tttgggaaada agctgaagga~~
 ttaaagtgtg tcctttgtct gtccatatcc taggttcaa ~~acatgttct tggacttct~~



33/240

pDEST1 6464 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
216..257		Trc promoter
397..273		attR1
647..1306		CmR
1426..1510		inactivated ccdA
1648..1953		ccdB
1994..2118		attR2
2598..3503		ampR
4104..4264		ori
4504..4941		flori (f1 intergenic region)
5340..6420		lacIq

1	GTTTGACAGC	TTATCATCGA	CTGCACGGTG	CACCAATGCT	TCTGGCGTCA	GGCAGCCATC
61	GGAAGCTGTG	GTATGGCTGT	GCAGGTCGTA	AATCACTGCA	TAATTCGTGT	CGCTCAAGGC
121	GCACTCCCGT	TCTGGATAAT	GTTTTTTGCG	CCGACATCAT	AACGGTTCGT	GCAAATATTC
181	TGAAATGAGC	TGTTGACAAT	TAATCATCCG	GTCCGTATAA	TCTGTGGAAT	TGTGAGCGGG
241	ATAACAATTT	CATCGCGAGG	TACCAAGCTA	TCACAAGTTT	GTACAAAAAA	GCTGAACGAG
301	AAACGTAAAA	TGATATAAAT	ATCAATATAT	TAAATTAGAT	TTTGCATAAA	AAACAGACTA
361	CATAATACTG	TAAAACACAA	CATATCCAGT	CACTATGGCG	GCCGCTAAGT	TGGCAGCATC
421	ACCCGACGCA	CTTTGCGCCG	AATAAATACC	TGTGACGGAA	GATCACTTCG	CAGAATAAAT
481	AAATCCTGGT	GTCCCTGTTG	ATACCGGAA	GCCCTGGGCC	AACTTTTGGC	GAAAAATGAGA
541	CGTTGATCGG	CACGTAAGAG	GTTCCAACCT	TCACCATAAT	GAAATAAGAT	CACTACCGGG
601	CGTATTTTTT	GAGTTATCGA	GATTTTCAGG	AGCTAAGGAA	GCTAAAATGG	AGAAAAAAT
661	CACTGGATAT	ACCACCGTTG	ATATATCCCA	ATGGCATCGT	AAAGAACATT	TTGAGGCATT
721	TCAGTCAGTT	GCTCAATGTA	CCTATAACCA	GACCGTTCAG	CTGGATATTA	CGGCCTTTTT
781	AAAGACCGTA	AAGAAAAATA	AGCACAAAGT	TTATCCGGCC	TTTATTACCA	TTCTTGCCCC
841	CCTGATGAAT	GCTCATCCGG	AATTCCGTAT	GGCAATGAAA	GACGGTGAGC	TGGTGATATG
901	GGATAGTGTT	CACCCTTGTT	ACACCGTTTT	CCATGAGCAA	ACTGAAACGT	TTTCATCGCT
961	CTGGAGTGAA	TACCACGACG	ATTTCCGGCA	GTTTCTACAC	ATATATTTCG	AAGATGTGGC
1021	GTGTTACGGT	GAAAACCTGG	CCTATTTCCC	TAAAGGGTTT	ATTGAGAATA	TGTTTTTCGT
1081	CTCAGCCAAT	CCCTGGGTGA	GTTTCACCAG	TTTTGATTTA	AACGTGGCCA	ATATGGACAA
1141	CTTCTTCGCC	CCCGTTTTCA	CCATGGGCAA	ATATTATACG	CAAGGCGACA	AGGTGCTGAT
1201	GCCGCTGGCG	ATTCAGGTTT	ATCATGCCGT	CTGTGATGGC	TTCCATGTCT	GCAGAATGCT
1261	TAATGAATTA	CAACAGTACT	GCGATGAGTG	GCAGGGCGGG	GCGTAAACGC	GTGGATCCGG
1321	CTTACTAAAA	GCCAGATAAC	AGTATGCGTA	TTTGCGCGCT	GATTTTTTGC	GTATAAGAAAT
1381	ATATACTGAT	ATGTATACCC	GAAGTATGTC	AAAAAGAGGT	GTGCTATGAA	GCAGCGTATT
1441	ACAGTGACAG	TTGACAGCGA	CAGCTATCAG	TTGCTCAAGG	CATATATGAT	GTCAATATCT
1501	CCGGTCTGGT	AAGCACAAAC	ATGCAGAATG	AAGCCCGTCG	TCTGCGTGCC	GAACGCTGGA
1561	AAGCGGAAAA	TCAGGAAGGG	ATGGCTGAGG	TCGCCCCGTT	TATTGAAATG	AACGGCTCTT
1621	TTGCTGACGA	GAACAGGGAC	TGGTGAAATG	CAGTTTAAAG	TTTACACCTA	TAAAAGAGAG
1681	AGCCGTTATC	GTCTGTTTGT	GGATGTACAG	AGTGATATTA	TTGACACGCC	CGGGCGACGG
1741	ATGGTGATCC	CCCTGGCCAG	TGCACGTCTG	CTGTACAGATA	AAGTCTCCCG	TGAACCTTAC
1801	CCGGTGGTGC	ATATCGGGGA	TGAAAGCTGG	CGCATGATGA	CCACCGATAT	GGCCAGTGTG
1861	CCGGTCTCCG	TTATCGGGGA	AGAACTGGCT	GATCTCAGCC	ACCGCGAAAA	TGACATCAAA
1921	AACGCCATTA	ACCTGATGTT	CTGGGGAATA	TAAATGTCTG	GCTCCCTTAT	ACACAGCCAG
1981	TCTGCAGGTC	GACCATAGTG	ACTGGATATG	TTGTGTTTTA	CAGTATTATG	TAGTCTGTTT
2041	TTTATGCAAA	ATCTAATTTA	ATATATTGAT	ATTTATATCA	TTTTACGTTT	CTCGTTCAGC
2101	TTTCTTGATC	AAAGTGGTGA	TAGCTTGGCT	GTTTTGGCGG	ATGAGAGAAG	ATTTTCAGCC
2161	TGATACAGAT	TAAATCAGAA	CGCAGAAGCG	GTCTGATAAA	ACAGAAATTT	CCTGGCGGCA
2221	GTAGCGCGGT	GGTCCCACCT	GACCCCATGC	CGAACTCAGA	AGTGAAACGC	CGTAGCGCCG
2281	ATGCTAGTGT	GGGGTCTCCC	CATGCGAGAG	TAGGGAACCT	CCAGGCATCA	AATAAAACGA
2341	AAGGCTCAGT	CGAAAGACTG	GGCCTTTCGT	TTTATCTGTT	GTTTGTCTGT	GAACGCTCTC
2401	CTGAGTAGGA	CAAAATCCGCC	GGGAGCGGAT	TTGAACGTTG	CGAAGCAACG	GCCCCGAGGG
2461	TGGCGGGCAG	GACGCCCCGC	ATAAACTGCC	AGGCATCAAA	TTAAGCAGAA	GGCCATCCTG
2521	ACGGATGGCC	TTTTTGCGTT	TCTACAAACT	CTTTTTGTTT	ATTTTCTTAA	ATACATTCAA-

FIGURE 21B

34/240

```

2581 ATATGTATCC GCTCATGAGA CAATAACCCT GATAAATGCT TCAATAATAT TGAAAAAGGA
2641 AGACTATGAG TATTCAACAT TTCCGTGTGCG CCCTTATTCC CTTTTTTGCG GCATTTTGCC
2701 TTCCTGTTTT TGCTCACCCA GAAACGCTGG TGAAAGTAAA AGATGCTGAA GATCAGTTGG
2761 GTGCACGAGT GGGTTACATC GAACTGGATC TCAACAGCGG TAAGATCCTT GAGASTTTTC
2821 GCCCCGAAGA ACGTTTTTCCA ATGATGAGCA CTTTTAAAGT TCTGCTATGT GCGCGGTAT
2881 TATCCCGTGT TGACGCCGGG CAAGAGCAAC TCGGTCGCCG CATACACTAT TCTCAGAAATG
2941 ACTTGGTTGA GTACTACCA GTACAGAAA AGCATCTTAC GGATGGCATG ACAGTAAGAG
3001 AATTATGCAG TGCTGCCATA ACCATGAGTG ATAACACTGC GGCCAACTTA CTTCTGACAA
3061 CGATCGGAGG ACCGAAGGAG CTAACCGCTT TTTTGCACAA CATGGGGGAT CATGTAATC
3121 GCCTTGATCG TTGGGAACCG GAGCTGAATG AAGCCATACC AAACGACGAG CGTGACACCA
3181 CGATGCCTAC AGCAATGGCA ACAACGTTGC GCAAACCTATT AACTGGCGAA CTACTTACTC
3241 TAGCTTCCCC GCAACAATTA ATAGACTGGA TGGAGGCGGA TAAAGTTGCA GGACCACTTC
3301 TGCGCTCGGC CCTTCCGGCT GGCTGGTTTA TTGCTGATAA ATCTGGAGCC GGTGAGCGTG
3361 GGTCTCGCGG TATCATTGCA GCACTGGGGC CAGATGGTAA GCCCTCCCGT ATCGTAGTTA
3421 TCTTACGAGC GGGGAGTCAG GCAACTATGG ATGAACGAAA TAGACAGATC GCTGAGATAG
3481 GTGCCTCACT GATTAAGCAT TGGTAACTGT CAGACCAAGT TTACTCATAT TACTTTTAGA
3541 TTGATTTAAA ACTTCATTTT TAATTTAAAA GGATCTAGGT GAAGATCCTT TTTGATAATC
3601 TCATGACCAA AATCCCTTAA CGTGAGTTTT CGTTCCTACTG AGCGTCAGAC CCCGTAGAAA
3661 AGATCAAAGG ATCTTCTTGA GATCCTTTTT TTCTGCGCGT AATCTGCTGC TTGCAAACAA
3721 AAAAACCACC GCTACCAGCG GTGGTTTGTT TGCCGGATCA AGAGCTACCA ACTCTTTTTC
3781 CGAAGGTAACT TGGCTTCAGC AGAGCGCAGA TACCAAATAC TGTCTTCTA GTGTAGCCGT
3841 AGTTAGGCCA CCACTTCAAG AACTCTGTAG CACCGCCTAC ATACCTCGCT CTGCTAATCC
3901 TGTTACCAGT GGCTGCTGCC AGTGGCGATA AGTCGTGTCT TACCGGGTTG GACTCAAGAC
3961 GATAGTTACC GGATAAGGCG CAGCGGTCGG GCTGAACGGG GGGTTCGTGC ACACAGCCCA
4021 GCTTGGAGCG AACGACCTAC ACCGAACTGA GATACCTACA GCGTGAGCTA TGAGAAAGCG
4081 CCACGCTTCC CGAAGGGAGA AAGCGGGACA GGTATCCGGT AAGCGGCAGG GTCGGAACAG
4141 GAGAGCGCAC GAGGGAGCTT CCAGGGGGAA ACGCCTGGTA TCTTTATAGT CCTGTGCGGT
4201 TTCGCCACCT CTGACTTGAG CGTCGATTTT TGTGATGCTC GTCAGGGGGG CGGAGCCTAT
4261 GGAAAAACGC CAGCAACGCG GCCTTTTTAC GGTTCCTGGC CTTTGTCTGG CCTTTTGCTC
4321 ACATGTTCTT TCCTGCGTTA TCCCCTGATT CTGTGGATAA CCGTATTACC GCCTTTGAGT
4381 GAGCTGATAC CGCTCGCCGC AGCCGAACGA CCGAGCGCAG CGAGTCAGTG AGCGAGGAAG
4441 CGGAAGAGCG CCTGATGCGG TATTTTCTCC TTACGCATCT GTGCGGTATT TCACACCGCA
4501 TAATTTTGTT AAAATTCCGG TTAATTTTTT GTTAAATCAG CTATTTTTTT AACCAATAGG
4561 CCGAAATCGG CAAAATCCCT TATAAATCAA AAGAATAGAC CGAGATAGGG TTGAGTGTG
4621 TTCCAGTTTG GAACAAGAGT CCACTATTAA AGAACGTGGA CTCCAACGTC AAAGGGCGAA
4681 AAACCGTCTA TCAGGGCGAT GGCCCACTAC GTGAACCATC ACCCTAATCA AGTTTTTTGG
4741 GGTCGAGGTG CCGTAAAGCA CTAAATCGGA ACCCTAAAGG GAGCCCCCGA TTTAGAGCTT
4801 GACGGGGAAA GCCGGCGAAC GTGGCGAGAA AGGAAGGGAA GAAAGCGAAA GGAGCGGGCG
4861 CTAGGGCGCT GGCAAGTGTA GCGGTCACGC TGCGCGTAAC CACCACACCC GCCGCGCTTA
4921 ATGCGCCGCT ACAGGGCGCG TCCATTGCGC ATTACAGGCTG CTATGGTGCA CTCTCAGTAC
4981 AATCTGCTCT GATGCCGCAT AGTTAAGCCA GTACCAGTCA CGTAGCGATA TCGGAGTGTA
5041 TACACTCCGC TATCGCTACG TGAATGGGTC ATGGCTGCGC CCCGACACCC GCCAACACCC
5101 GCTGACGCGC CCTGACGGGC TTGTCTGCTC CCGGCATCCG CTTACAGACA AGCTGTGACC
5161 GTCTCCGGGA GCTGCATGTG TCAGAGGTTT TCACCGTCAT CACCGAAACG CGCGAGGCAG
5221 CAGATCAATT CGCGCGCGAA GGCGAAGCGG CATGCATTTA CGTTGACACC ATCGAATGGT
5281 GCAAAACCTT TCGCGGTATG GCATGATAGC GCGCGGAAGA GAGTCAATTC AGGGTGGTGA
5341 ATGTGAAACC AGTAACGTTA TACGATGTCG CAGAGTATGC CCGTGTCTCT TATCAGACCG
5401 TTTCCCGCGT GGTGAACGAG GCCAGCCACG TTTCTGCGAA AACCGGGGAA AAAGTGGAAG
5461 CGGCGATGCG GGAGCTGAAT TACATTTCCA ACCCGGTGGC ACAACAAGT GCGGGCAAAC
5521 AGTCGTTGCT GATTGGCGTT GCCACCTCCA GTCTGGCCCT GCACGCGCGG TCGCAAATTG
5581 TCGCGGCGAT TAAATCTCGC GCCGATCAAC TGGGTGCCAG CGTGGTGGTG TCGATGGTAG
5641 AACGAAGCGG CGTCGAAGCC TGTAAGCGG CGGTGCACAA TCTTCTCGCG CAACGCGTCA
5701 GTGGGCTGAT CATTAACCTAT CCGCTGGATG ACCAGGATGC CATTGCTGTG GAAGCTGCCT
5761 GCACTAATGT TCCGGCGTTA TTTCTTGATG TCTCTGACCA GACACCCATC AACAGTATTA
5821 TTTTCTCCCA TGAAGACGGT ACGCGACTGG GCGTGGAGCA TCTGGTCCGA TTGGGTCAAC
5881 AGCAAATCGC GCTGTTAGCG GCGCCATTA GTTCTGTCTC GCGCGCTCTG CGTCTGGCTG
5941 GCTGGCATAA ATATCTCACT CGCAATCAA TTCAGCCGAT AGCGGAACGG GAAGGCGACT
6001 GGAGTGCCAT GTCCGGTTTT CAACAAACCA TGCAAATGCT GAATGAGGGC ATCGTTCCCA-

```

FIGURE 21C

35/240

6061 CTGCGATGCT GGTGCGCAAC GATCAGATGG CGCTGGGCGC AATGCGCGCC ATTACCGAGT
6121 CCGGGCTGCG CGTTGGTGCG GATATCTCGG TAGTGGGATA CGACGATACC GAAGACAGCT
6181 CATGTTATAT CCCGCCGTTA ACCACCATCA AACAGGATTT TCGCCTGCTG GGGCAAACCA
6241 GCGTGGACCG CTTGCTGCAA CTCTCTCAGG GCCAGGCGGT GAAGGGCAAT CAGCTGTTGC
6301 CCGTCTCACT GGTGAAAAGA AAAACCACCC TGGCACCCAA TACGCAAACC GCCTCTCCCC
6361 GCGCGTTGGC CGATTCATTA ATGCAGCTGG CACGACAGGT TTCCCGACTG GAAAGCGGGC
6421 AGTGAGCGCA ACGCAATTAA TGTGAGTTAG CGCGAATTGA TCTG

FIGURE 21D

36/240

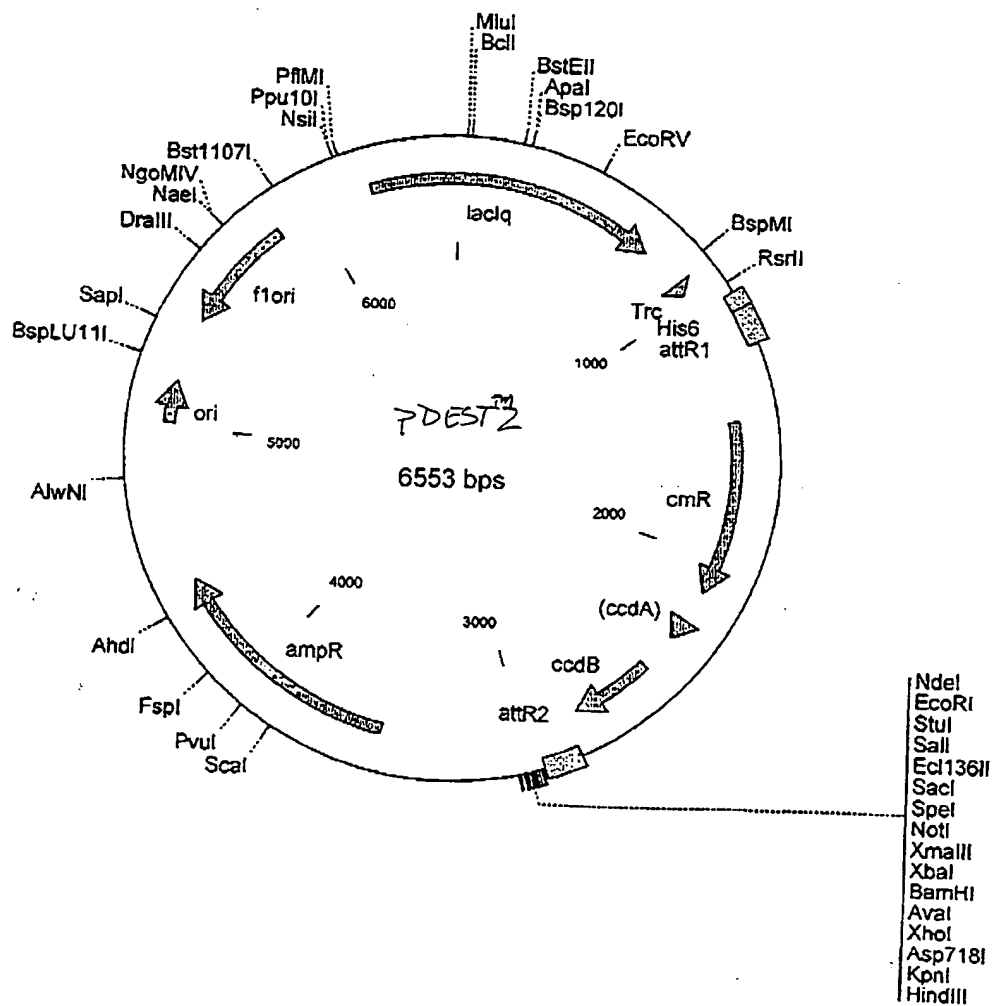
Figure 22A: pDEST2

His6 fusions in E. coli

970 aat att ctg aaa tga gct ggt gac aat ⁻³⁵ tta tca tcc ggt ccg ⁻¹⁰ tat aat ctg
 tta taa gac ttt act cga caa ctg tta att agt agg cca ggc ata tta gac

1021 tgg aat ^{RNA} tgt gag cgg ata aca att tca cac agg aaa cag acc Met Ser Tyr
 acc tta aca ctc gcc tat tgt taa agt gtg tcc ttt gtc tgg tac agc atg

1072 tac cat cac cat cac cat ^{His His His His His His His} ggt att ^{Gly Ile} aca agt ttg ^{Trp Ser Pst} taa aaa gct gaa ^{attR1}
 atg gta gtg gta gtg gta gtg ccg tag tgt tca aac atg ttt ctt cga cgt



37/240

pDEST2 6553 bp

<u>Location (Base Nos.)</u>	<u>Gene Encoded</u>
912..962	Trc
1223..1009	attR1
1473..2132	CmR
2252..2336	inactivated ccdA
2474..2779	ccdB
2820..2944	attR2
3509..4414	ampR
5015..5175	ori
5415..5852	flori (f1 intergenic region)
6225..752	lacIq

```

1  GCGGGTGCAC AATCTTCTCG CGCAACGCGT CAGTGGGCTG ATCATTAACT ATCCGCTGGA
61  TGACCAGGAT GCCATTGCTG TGGAAGCTGC CTGCACTAAT GTTCCGGCGT TATTTCTTGA
121 TGTCTCTGAC CAGACACCCA TCAACAGTAT TATTTTCTCC CATGAAGACG GTACGCGACT
181 GGGCGTGGAG CATCTGGTCG CATTGGGTCA CCAGCAAATC GCGCTGTAG CGGGCCATT
241 AAGTTCTGTC TCGGCGCGTC TGCGTCTGGC TGGCTGGCAT AAATATCTCA CTCGCAATCA
301 AATTCAGCCG ATAGCGGAAC GGAAGGCGA CTGGAGTGCC ATGTCCGGTT TTCAACAAAC
361 CATGCAAATG CTGAATGAGG GCATCGTTCC CACTGCGATG CTGGTTGCCA ACGATCAGAT
421 GCGGCTGGGC GCAATGCGCG CCATTACCGA GTCCGGGCTG CGCGTTGGTG CGGATATCTC
481 GGTAGTGGGA TACGACGATA CCGAAGACAG CTCATGTTAT ATCCCGCCGT CAACCACCAT
541 CAAACAGGAT TTTCGCCCTG TGGGGCAAAC CAGCGTGGAC CGCTTGCTGC AACTCTCTCA
601 GGGCCAGGCG GTGAAGGGCA ATCAGCTGTT GCCCGTCTCA CTGGTGAAAA GAAAAACCAC
661 CCTGGCACCC AATACGCAAA CCGCCTCTCC CCGCGCGTTG GCCGATTCAT TAATGCAGCT
721 GGCACGACAG GTTTCGCCGAC TGGAAAGCGG GCAGTGAGCG CAACGCAATT AATGTGAGTT
781 AGCGCGAATT GATCTGGTTT GACAGCTTAT CATCGACTGC ACGGTGCACC AATGCTTCTG
841 GCGTCAGGCA GCCATCGGAA GCTGTGGTAT GGTGTGTCAG GTCGTAATC ACTGCATAAT
901 TCGTGTGCTC CAAGGCGCAC TCCCGTCTG GATAATGTTT TTGCGCCGA CATCATAACG
961 GTTCTGCGAA ATATTCTGAA ATGAGCTGTT GACAATTAAT CATCCGGTCC GTATAATCTG
1021 TGGAATTGTG AGCGGATAAC AATTTACAC AGGAAACAGA CCATGTCGTA CTACCATCAC
1081 CATCACCATC ACGGCATCAC AAGTTTGTAC AAAAAAGCTG AACGAGAAAC GTAAATGAT
1141 ATAAATATCA ATATATTAAT TTAGATTTTG CATAAAAAAC AGACTACATA ATACTGTAAA
1201 ACACAACATA TCCAGTCACT ATGGCGGCCG CTAAGTTGGC AGCATCAGCC GACGCACTTT
1261 GCGCGGAATA AATACCTGTG ACGGAAGATC ACTTCGCAGA ATAAATAAAT CCTGGTGTCC
1321 CTGTTGATAC CGGGAAGCCC TGGGCCAACT TTTGGCGAAA ATGAGACGTT GATCGGCACG
1381 TAAGAGGTTT CAACTTTCAC CATAATGAAA TAAGATCACT ACCGGGCGTA TTTTGTGAGT
1441 TATCGAGATT TTCAGGAGCT AAGGAAGCTA AAATGGAGAA AAAAATCACT GGATATACCA
1501 CCGTTGATAT ATCCCAATGG CATCGTAAAG AACATTTTGA GGCATTTTCA TCAGTTGCTC
1561 AATGTACCTA TAACCAGACC GTTCAGCTGG ATATTACGGC CTTTTTAAAG ACCGTAAAGA
1621 AAAATAAGCA CAAGTTTAT CCGGCCTTTA TTCACATTCT TGCCCGCCTG ATGAATGCTC
1681 ATCCGGAATT CCGTATGGCA ATGAAAGACG GTGAGCTGGT GATATGGGAT AGTGTTTACC
1741 CTTGTTACAC CGTTTCCAT GAGCAAACCT AAACGTTTTT ATCGCTCTGG AGTGAATACC
1801 ACGACGATTT CCGGCAGTTT CTACACATAT ATTGCAAGA TGTGGCGTGT TACGGTGAAA
1861 ACCTGGCCTA TTTCCCTAAA GGGTTTATTG AGAATATGTT TTTGCTCTCA GCCAATCCCT
1921 GGGTGAGTTT CACCAAGTTT GATTTAAACG TGGCCAATAT GGACAACTTC TTCGCCCCCG
1981 TTTTACCAT GGGCAAATAT TATACGCAAG GCGACAAGGT GCTGATGCCG CTGGCGATTC
2041 AGGTTTCATCA TGCCGTCTGT GATGGCTTCC ATGTCGGCAG AATGCTTAAT GAATTACAAC
2101 AGTACTGCGA TGAGTGGCAG GCGGGGCGT AAACGCGTGG ATCCGGCTTA CTAAAGCCA
2161 GATAACAGTA TGCGTATTG CGCGCTGATT TTTGCGGTAT AAGAATATAT ATGATATGAT
2221 ATACCCGAAG TATGTCAAAA AGAGGTGTGC TATGAAGCAG CGTATTACAG TGACAGTTGA
2281 CAGCGACAGC TATCAGTTGC TCAAGGCATA TATGATGTCA ATATCTCCGG TCTGGTAAGC
2341 ACAACCATGC AGAATGAAGC CCGTCGCTG CGTGCCGAAC GCTGGAAAGC GGAAATCAG
2401 GAAGGGATGG CTGAGGTGCG CCGGTTTATT GAAATGAACG GCTCTTTTGC TGACGAGAAC
2461 AGGACTGGT GAAATGCAGT TTAAGTTTAA CACCTATAAA AGAGAGAGCC GTTATCGTCT
2521 GTTGTGGAT GTACAGAGTG ATATTATTGA CACGCCCGG CGACGGATGG TGATCCCCCT-

```

FIGURE 22B

38/240

```

2581 GGCCAGTGCA CGTCTGCTGT CAGATAAAGT CTCCCGTGAA CTTTACCCGG TGGTGCATAT
2641 CGGGGATGAA AGCTGGCGCA TGATGACCAC CGATATGGCC AGTGTGCCGG TCTCCGTTAT
2701 CGGGGAAGAA GTGGCTGATC TCAGCCACCG CGAAAATGAC ATCAAAAACG CCATTAACCT
2761 GATGTTCTGG GGAATATAAA TGTCAGGCTC CCTTATACAC AGCCAGTCTG CAGGTGCGACC
2821 ATAGTGACTG GATATGTTGT GTTTTACAGT ATTATGTAAGT CTGTTTTTTA TGCAAAATCT
2881 AATTTAATAT ATTGATATTT ATATCATTTT ACGTTTCTCG TTCAGCTTTC TTGTACAAAG
2941 TGGTGATGCC CATATGGGAA TTCAAAGGCC TACGTCGACG AGCTCACTAG TCGCCGCCGC
3001 TTCTAGAGGA TCCCTCGAGG CATGCGGTAC CAAGCTTGGC TGTTTTGGCG GATGAGAGAA
3061 GATTTTTCAGC CTGATACAGA TTAAATCAGA ACGCAGAAGC GGTCTGATAA AACAGAATTT
3121 GCCTGGCGGC AGTAGCGCGG TGGTCCCACC TGACCCCATG CCGAACTCAG AAGTGAAACG
3181 CCGTAGCGCC GATGGTAGTG TGGGGTCTCC CCAATGCGAGA GTAGGGAAGT GCCAGGCATC
3241 AAATAAAACG AAAGGCTCAG TCGAAAGACT GGGCCTTTCG TTTTATCTGT TGTTTGTCGG
3301 TGAACGCTCT CTGAGTAGG ACAAATCCGC CGGGAGCGGA TTTGAACGTT GCGAAGCAAC
3361 GGGCCGAGG GTGGCGGGCA GGACGCCCGC CATAACTGCG CAGGCATCAA ATTAAGCAGA
3421 AGGCCATCCT GACGGATGGC CTTTTTGGCT TTCTACAAAC TCTTTTGTGT TATTTTCTA
3481 AATACATTCA AATATGTATC CGCTCATGAG ACAATAACCC TGATAAATGC TTCAATAATA
3541 TTGAAAAGG AAGAGTATGA GTATTCAACA TTTCCGTGTC GCCCTTATTC CTTTTTTGTC
3601 GGCATTTTGC CTTCTGTTT TTGCTCACC AGAAACGCTG GTGAAAGTAA AAGATGCTGA
3661 AGATCAGTTG GGTGCACGAG TGGGTACAT CGAACTGGAT CTCAACAGCG GTAAGATCCT
3721 TGAGAGTTTT CGCCCCGAG AACGTTTTC AATGATGAGC ACTTTTAAAG TTCTGCTATG
3781 TGGCGCGGTA TTATCCCGTG TTGACGCCGG GCAAGAGCAA CTCGGTCGCG GCATACACTA
3841 TTCTCAGAAT GACTTGGTTG AGTACTACC AGTCACAGAA AAGCATCTTA CGGATGGCAT
3901 GACAGTAAGA GAATTATGCA GTGCTGCCAT AACCATGAGT GATAAACTG CGGCCAACTT
3961 ACTTCTGACA ACGATCGGAG GACCGAAGGA GCTAACCCTG TTTTGCACA ACATGGGGGA
4021 TCATGTAACT CGCCTTGATC GTTGGGAACC GGAGCTGAAT GAAGCCATAC CAAACGACGA
4081 GCGTGACACC ACGATGCCTA CAGCAATGGC AACAACTTG CGCAAATAT TAACCTGGCGA
4141 ACTACTTACT CTAGCTTCCC GGCAACAATT AATAGACTGG ATGGAGGCGG ATAAAGTTGC
4201 AGGACCACTT CTGCGCTCGG CCCTTCCGGC TGGCTGGTTT ATTGCTGATA AATCTGGAGC
4261 CGGTGAGCGT GGGTCTCGCG GTATCATTGC AGCACTGGGG CCAGATGGTA AGCCTCCCCG
4321 TATCGTAGTT ATCTACACGA CGGGGAGTCA GGCAACTATG GATGAACGAA ATAGACAGAT
4381 CGCTGAGATA GGTGCCTCAC TGATTAAAGCA TTGGTAACTG TCAGACCAAG TTTACTCATA
4441 TATACTTAG ATTGATTTAA AACTTCATTT TTAATTTAAA AGGATCTAGG TGAAGATCCT
4501 TTTTGATAAT CTCATGACCA AAATCCCTTA ACGTGAGTTT TCGTTCCTACT GAGCGTCAGA
4561 CCCCGTAGAA AAGATCAAAG GATCTTCTTG AGATCCTTTT TTTCTGCGCG TAATCTGCTG
4621 CTTGCAACA AAAAAACCAC CGCTACCAGC GGTGGTTTGT TTGCCGATC AAGAGCTACC
4681 AACTCTTTTT CCGAAGGTAA CTGGCTTCAG CAGAGCGCAG ATACCAAATA CTGTCTTCT
4741 AGTGTAGCCG TAGTTAGGCC ACCACTTCAA GAACTCTGTA GCACCGCCTA CATACCTCGC
4801 TCTGTAAATC CTGTTACCAG TGGCTGCTGC CAGTGGCGAT AAGTCGTGTC TTACCGGGTT
4861 GGACTCAAGA CGATAGTTAC CGGATAAGGC GCAGCGGTCG GGCTGAACGG GGGGTTCTGT
4921 CACACAGCCC AGCTTGGAGC GAACGACCTA CACCGAAGT AGATACCTAC AGCGTGAGCT
4981 ATGAGAAAGC GCCACGCTTC CCGAAGGGAG AAAGGCGGAC AGGTATCCGG TAAGCGGCAG
5041 GGTGGAACA GGAGAGCGCA CGAGGGAGCT TCCAGGGGGA AACGCCTGGT ATCTTTATAG
5101 TCCTGTGGG TTTGCCACC TCTGACTTGA GCGTCGATTT TTGTGATGCT CGTCAGGGGG
5161 GCGGAGCCTA TGGAAAACG CCAGCAACGC GGCCTTTTTA CGGTTCCTGG CTTTTTGCTG
5221 GCCTTTTGCT CACATGTTCT TTCCTGCGTT ATCCCCTGAT TCTGTGGATA ACCGTATTAC
5281 CGCCTTTGAG TGAGCTGATA CCGCTCGCCG CAGCCGAACG ACCGAGCGCA GCGAGTCAGT
5341 GAGCGAGGAA GCGGAAGAGC GCCTGATGCG GTATTTTCTC CTTACGCATC TGTGCGGTAT
5401 TTCACACCGC ATAATTTTGT TAAAATTCGC GTTAAATTTT TGTTAAATCA GCTCATTTTT
5461 TAACCAATAG GCCGAAATCG GCAAATCCC TTATAAATCA AAAGAATAGA CCGAGATAGG
5521 GTTGAGTGTT GTTCCAGTTT GGAACAAGAG TCCACTATTA AAGAACGTGG ACTCCAACGT
5581 CAAAGGCGA AAAACCGTCT ATCAGGGCGA TGGCCCACTA CGTGAACCAT CACCCTAATC
5641 AAGTTTTTTG GGTGCGAGGT GCCGTAAAGC ACTAAATCGG AACCCTAAAG GGAGCCCCCG
5701 ATTTAGAGCT TGACGGGGAA AGCCGCGGAA CGTGGCGAGA AAGGAAGGGA AGAAAGCGAA
5761 AGGAGCGGGC GCTAGGGCGC TGGCAAGTGT AGCGGTCACG CTGCGCGTAA CCACCACACC
5821 CGCCGCGCTT AATGCGCCGC TACAGGGGCG GTCCCATTCG CCATTCAGCG TGCTATGGTG
5881 CACTCTCAGT ACAATCTGCT CTGATGCCGC ATAGTTAAGC CAGTATACAC TCCGCTATCG
5941 CTACGTGACT GGGTCATGGC TGCGCCCCGA CACCCGCCAA CACCCGCTGA CGCGCCCTGA
6001 CGGGCTTGTC TGCTCCCGGC ATCCGCTTAC AGACAAGCTG TGACCGTCTC CGGGAGCTGC

```

FIGURE 22C

6061 ATGTGTCAGA GGTTTTACC GTCATCACCG AACGCGCGA GGCAGCAGAT CAATTCGCGC
6121 GCGAAGGCGA AGCGGCATGC ATTTACGTTG ACACCATCGA ATGGTGCAAA ACCTTTCGCG
6181 GTATGGCATG ATAGCGCCCG GAAGAGAGTC AATTCAGGGT GGTGAATGTG AAACCAGTAA
6241 CGTTATACGA TGTCGCAGAG TATGCCGGTG TCTCTTATCA GACCGTTTCC CGCGTGGTGA
6301 ACCAGGCCAG CCACGTTTCT GCGAAAACGC GGGAAAAAGT GGAAGCGGCG ATGGCGGAGC
6361 TGAATTACAT TCCCAACCGC GTGGCACAAC AACTGGCGGG CAAACAGTCG TTGCTGATTG
6421 GCGTTGCCAC CTCCAGTCTG GCCCTGCACG CGCCGTCGCA AATTGTCGCG GCGATTAAAT
6481 CTCGCGCCGA TCAACTGGGT GCCAGCGTGG TGGTGTGAT GGTAGAACGA AGCGGCGTCG
6541 AAGCCTGTAA AGC

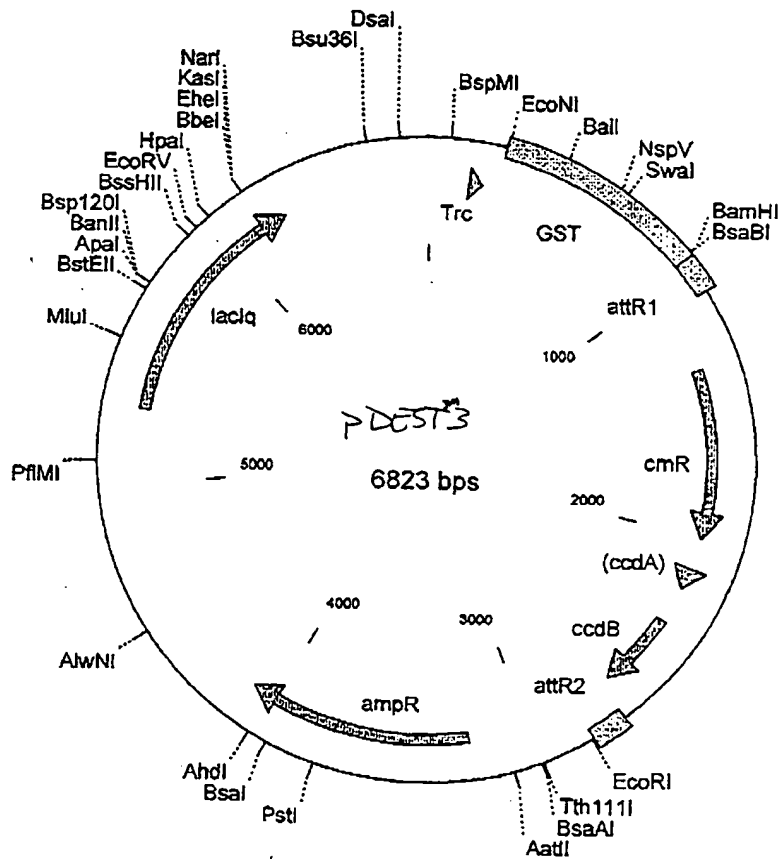
40/240

Figure 23A: pDEST3

GST fusions in E. coli

154 egg ttc tgg caa ata ttc tga aat gag ctg ⁻³⁵ ttg aca att ^{Trc promoter} aat cat cgg ctc
 gcc aag acc gtt tat aag act tta ctc gac ⁻¹⁰ aac tgt taa tta gta gcc gag
 205 gca taa tgt gtg gaa ttg tga gcg gat aac aat ttc aca cag gaa aca gta
 cat att aca cac ctt aac act cgc cta ttg tta aag tgt gtc ctt tgt cat
 256 ttc atg tcc cct ata cta ggt tat tgg aaa att aag ggc ctt gtg caa ccc
 aag tac agg gga tat gat cca ata acc ttt taa ttc ccg gaa cac gtt ggg

" GST → R G S R R A S V G S P S T S
 919 ctg gtt ccg cgt gga tct cgt cgt gca tct gtt gga tcc cca tca ^{attR1} aca agt
 gac caa ggc gca cct aga gca gca cgt aga caa cct agg ggt agt ^{attR2} tgt tca
 970 ~~tgg ttc aaa gct gaa cga gaa acg taa aat gat ata aat acc aat ata~~
~~aac atg ttt ttc cga cgt gct cct tgc att tta cta tat tta tag tta tat~~



41/240

pDEST3 6823 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
150..200		Trc
1087..963		attR1
1337..1996		CmR
2116..2200		inactivated ccdA
2338..2643		ccdB
2684..2808		attR2
3231..4091		ampR
5295..6254		lacIq
1	ACGTTATCGA CTGCACGGTG CACCAATGCT TCTGGCGTCA GGCAGCCATC GGAAGCTGTG	
61	GTATGGCTGT GCAGGTCGTA AATCACTGCA TAATTCGTGT CGCTCAAGGC GCACTCCCCT	
121	TCTGGATAAT GTTTTGTGCG CCGACATCAT AACGGTTCCTG GCAAATATTC TGAATGAGC	
181	TGTTGACAAT TAATCATCGG CTCGTATAAT GTGTGGAATT GTGAGCGGAT AACAAATTTCA	
241	CACAGGAAAC AGTATTCATG TCCCCTATAC TAGGTTATTG GAAAATTAAG GGCCTTGTGC	
301	AACCCACTCG ACTTCTTTTG GAATATCTTG AAGAAAAATA TGAAGAGCAT TTGTATGAGC	
361	GCGATGAAGG TGATAAATGG CGAAACAAAA AGTTTGAATT GGGTTTGGAG TTTCCCAATC	
421	TTCTTATTA TATTGATGGT GATGTTAAAT TAACACAGTC TATGGCCATC ATACGTTATA	
481	TAGCTGACAA GCACAACATG TTGGGTGGTT GTCCAAAAGA GCGTGCAGAG ATTTCAATGC	
541	TTGAAAGGAGC GGTTTTGGAT ATTAGATACG GTGTTTCGAG AATTGCATAT AGTAAAGACT	
601	TTGAAACTCT CAAAGTTGAT TTTCTTAGCA AGCTACCTGA AATGCTGAAA ATGTTCGAAG	
661	ATCGTTTATG TCATAAAACA TATTTAAATG GTGATCATGT AACCCATCCT GACTTCATGT	
721	TGTATGACGC TCTTGATGTT GTTTTATACA TGGACCCAAT GTGCCTGGAT GCGTCCCAA	
781	AATTAGTTTG TTTTAAAAAA CGTATTGAAG CTATCCCACA AATTGATAAG TACTTGAAAT	
841	CCAGCAAGTA TATAGCATGG CCTTTGCAGG GCTGGCAAGC CACGTTTGGT GGTGGCGACC	
901	ATCCTCCAAA ATCGGATCTG GTTCCGCGTG GATCTCGTCG TGCATCTGTT GGATCCCCAT	
961	CAACAAGTTT GTACAAAAAA GCTGAACGAG AAACGTAAAA TGATATAAAT ATCAATATAT	
1021	TAAATTAGAT TTTGCATAAA AAACAGACTA CATAATACTG TAAAACACAA CATATCCAGT	
1081	CACTATGGCG GCCGCTAAGT TGGCAGCATC ACCCGACGCA CTTTGCGCCG AATAAATACC	
1141	TGTGACGGAA GATCACTTCG CAGAATAAAT AAATCCTGGT GTCCCTGTTG ATACCGGGA	
1201	GCCCTGGGCC AACTTTTGGC GAAAATGAGA CGTTGATCGG CACGTAAGAG GTTCCAACCT	
1261	TCACCATAAT GAAATAAGAT CACTACCGGG CGTATTTTTT GAGTTATCGA GATTTTCAGG	
1321	AGCTAAGGAA GCTAAAATGG AGAAAAAAT CACTGGATAT ACCACCGTTG ATATATCCCA	
1381	ATGGCATCGT AAAGAACATT TTGAGGCATT TCAGTCAGTT GCTCAATGTA CCTATAACCA	
1441	GACCGTTCAG CTGGATATTA CGGCCTTTT AAAGACCGTA AAGAAAAATA AGCACAAGTT	
1501	TTATCCGGCC TTTATTCACA TTCTTGCCCG CCTGATGAAT GCTCATCCGG AATTCGGTAT	
1561	GGCAATGAAA GACGCTGAGC TGGTGATATG GGATAGTGTT CACCCTTGTT ACACCGTTTT	
1621	CCATGAGCAA ACTGAAACGT TTTTCATCGT CTGGAGTGAA TACCACGACG ATTTCCGGCA	
1681	GTTTCTACAC ATATATTGCG AAGATGTGGC GTGTTACGGT GAAAACCTGG CCTATTTCCC	
1741	TAAAGGGTTT ATTGAGAATA TGTTTTTCGT CTCAGCCAAT CCCTGGGTGA GTTTCACCAG	
1801	TTTTGATTTA AACGTGGCCA ATATGGACAA CTTCTTCGCC CCCGTTTTCA CCATGGGCAA	
1861	ATATTATACG CAAGGCGACA AGGTGCTGAT GCCGCTGGCG ATTCAGGTTT ATCATGCCGT	
1921	CTGTGATGGC TTCCATGTCG GCAGAATGCT TAATGAATTA CAACAGTACT GCGATGAGTG	
1981	GCAGGGCGGG GCGTAAAGAT CTGGATCCGG CTTACTAAAA GCCAGATAAC AGTATGCGTA	
2041	TTTGCGCGCT GATTTTTCG GTATAAGAA ATATACTGAT ATGTATACCC GAAGTATGTC	
2101	AAAAAGAGGT GTGCTATGAA GCAGCGTATT ACAGTGACAG TTGACAGCGA CAGCTATCAG	
2161	TTGCTCAAGG CATATATGAT GTCAATATCT CCGGTCTGGT AAGCACAACC ATGCAGAATG	
2221	AAGCCCGTCG TCTGCGTGCC GAACGCTGGA AAGCGGAAAA TCAGGAAGGG ATGGCTGAGG	
2281	TCGCCCCGTT TATTGAAATG AACGGCTCTT TTGCTGACGA GAACAGGGAC TGGTGAAATG	
2341	CAGTTTAAAG TTTACACCTA TAAAAGAGAG AGCCGTTATC GTCTGTTTGT GGATGTACAG	
2401	AGTGATATTA TTGACACGCC CGGGCGACGG ATGGTGATCC CCCTGGCCAG TGCACGTCTG	
2461	CTGTCAGATA AAGTCTCCCG TGAACTTTAC CCGGTGGTGC ATATCGGGGA TGAAGCTGG	
2521	CGCATGATGA CCACCGATAT GGCCAGTGTG CCGGTCTCCG TTATCGGGGA AGAAGTGGCT	
2581	GATCTCAGCC ACCGCGAAAA TGACATCAAA AACGCCATTA ACCTGATGTT CTGGGAATA	
2641	TAAATGTCAG GCTCCCTTAT ACACAGCCAG TCTGCAGGTC GACCATAGTG ACTGGATATG	

FIGURE 23B

42/240

```

2701 TTGTGTTTTA CAGTATTATG TAGTCTGTTT TTTATGCAAA ATCTAATTTA ATATATTGAT
2761 ATTTATATCA TTTTACGTTT CTCGTTTCAGC TTTCTTGTAAC AAAGTGGTTG ATGGGAATTC
2821 ATCGTGACTG ACTGACGATC TGCCTCGCGC GTTTCGGTGA TGACGGTGAA AACCTCTGAC
2881 ACATGCAGCT CCCGAGACG GTCACAGCTT GTCTGTAAGC GGATGCCGGG AGCAGACAAG
2941 CCCGTCAGGG CGCGTCAGCG GGTGTTGGCG GGTGTCGGGG CGCAGCCATG ACCCAGTCAC
3001 GTAGCGATAG CGGAGTGTAT AATTCTTGAA GACGAAAGGG CCTCGTGATA CGCCTATTTT
3061 TATAGGTTAA TGTATGATA ATAATGGTTT CTTAGACGTC AGGTGGCACT TTTCCGGGAA
3121 ATGTGCGCGG AACCCCTATT TGTATTATTT TCTAAATACA TTCAAATATG TATCCGCTCA
3181 TGAGACAATA ACCCTGATAA ATGCTTCAAT AATATTGAAA AAGGAAGAGT ATGAGTATTC
3241 AACATTTCGG TGTGCCCCCT ATTCCCTTTT TTGCGGCATT TTGCCCTCCT GTTTTGTCTC
3301 ACCCAGAAAC GCTGGTGAAA GTAAAAGATG CTGAAGATCA GTTGGGTGCA CGAGTGGGTT
3361 ACATCGAACT GGATCTCAAC AGCGGTAAAG TCTTTGAGAG TTTTCGCCCC GAAGAACGTT
3421 TTCCAATGAT GAGCACTTTT AAAGTTCTGC TATGTGGCGC GGTATTATCC CGTGTGACG
3481 CCGGGCAAGA GCAACTCGGT CGCCGCATAC ACTATTCTCA GAATGACTTG GTTGAGTACT
3541 CACCAGTCAC AGAAAAGCAT CTTACGGATG GCATGACAGT AAGAGAATTA TGCAGTGCTG
3601 CCATAACCAT GAGTGATAAC ACTGCGGCCA ACTTACTTCT GACAACGATC GGAGGACCGA
3661 AGGAGCTAAC CGCTTTTTTG CACAACATGG GGGATCATGT AACTCGCCTT GATCGTTGGG
3721 AACCAGAGCT GAATGAAGCC ATACCAAACG ACGAGCGTGA CACCACGATG CCTGCAGCAA
3781 TGGCAACAAC GTTGCGCAAA CTATTAAGTG GCGAACTACT TACTCTAGCT TCCCGGCAAC
3841 AATTAATAGA CTGGATGGAG GCGGATAAAG TTGCAGGACC ACTTCTGCGC TCGGCCCTTC
3901 CGGCTGGCTG GTTTATTGCT GATAAATCTG GAGCCGGTGA GCGTGGGTCT CGCGGTATCA
3961 TTGCAGCACT GGGGCCAGAT GGTAAGCCCT CCCGTATCGT AGTTATCTAC ACGACGGGGA
4021 GTCAGGCAAC TATGGATGAA CGAAATAGAC AGATCGCTGA GATAGGTGCC TCACTGATTA
4081 AGCATTGGTA ACTGTCAGAC CAAGTTTACT CATATATACT TTAGATTGAT TTAAAACCTC
4141 ATTTTAAATT TAAAAGGATC TAGGTGAAGA TCCTTTTTGA TAATCTCATG ACCAAAATCC
4201 CTTAACGTGA GTTTTCGTTT CACTGAGCGT CAGACCCCGT AGAAAAGATC AAAGGATCTT
4261 CTTGAGATCC TTTTTCCTG CGCGTAATCT GCTGCTTGCA AACAAAAAAA CCACCGCTAC
4321 CAGCGTGGT TTGTTTGCCG GATCAAGAGC TACCAACTCT TTTTCCGAAG GTAACCTGGC
4381 TCAGCAGAGC GCAGATACCA AATACTGTCC TTCTAGTGTA GCCGTAGTTA GGCCACCACT
4441 TCAAGAACTC TGTAGCACCG CCTACATACC TCGCTCTGCT AATCCTGTTA CCAGTGGCTG
4501 CTGCCAGTGG CGATAAGTCG TGTCTTACCG GGTGGGACTC AAGACGATAG TTACCGGATA
4561 AGGCGCAGCG GTCGGGCTGA ACGGGGGGTT CGTGACACACA GCCCAGCTTG GAGCGAACGA
4621 CCTACACCGA ACTGAGATAC CTACAGCGTG AGCTATGAGA AAGCGCCACG CTTCGCCAAG
4681 GGAGAAAGGC GGACAGGTAT CCGGTAAGCG GCAGGGTCGG AACAGGAGAG CGCAGGAGG
4741 AGCTTCCAGG GGGAAACGCC TGGTATCTTT ATAGTCCTGT CGGGTTTCGC CACCTCTGAC
4801 TTGAGCGTCG ATTTTGTGA TGCTCGTCAG GGGGGCGGAG CCTATGGAAA AACGCCAGCA
4861 ACGCGGCCCT TTTACGGTTC CTGGCCTTTT GCTGGCCTTT TGCTCACATG TTCTTTCTCTG
4921 CGTTATCCCC TGATTCTGTG GATAACCGTA TTACCGCCTT TGAGTGAGCT GATACCGCTC
4981 GCCGCAGCCG AACGACCGAG CGCAGCGAGT CAGTGAGCGA GGAAGCGGAA GAGCGCCTGA
5041 TGCGGTATTT TCTCCTTACG CATCTGTGCG GTATTTTACA CCGCATAAAT TCCGACACCA
5101 TCGAATGGTG CAAAACCTTT CGCGGTATGG CATGATAGCG CCCGGAAGAG AGTCAATTCA
5161 GGGTGGTGAA TGTGAAACCA GTAACGTTAT ACGATGTCGC AGAGTATGCC GGTGTCTCTT
5221 ATCAGACCGT TTCCCGCGTG GTGAACCAGG CCAGCCACGT TTCTGCGAAA ACGCGGAAA
5281 AAGTGGAAGC GCGGATGGCG GAGCTGAATT ACATTCCCAA CCGCGTGGCA CAACAACTGG
5341 CCGGCAACA GTCGTTGCTG ATTGGCGTTG CCACCTCCAG TCTGGCCCTG CACGCGCCGT
5401 CGCAAATTGT CGCGGCGATT AAATCTCGCG CCGATCAACT GGGTGCCAGC GTGGTGGTGT
5461 CGATGGTAGA ACGAAGCGGC GTCGAAGCCT GTAAAGCGGC GGTGCACAAT CTCTCGCGC
5521 AACGCGTCAG TGGGCTGATC ATTAAGTATC CGCTGGATGA CCAGGATGCC ATTGCTGTGG
5581 AAGCTGCCTG CACTAATGTT CGCGGCTTAT TTCTTGATGT CTCTGACCAG ACACCCATCA
5641 ACAGTATTAT TTTCTCCCAT GAAGACGGTA CGCGACTGGG CGTGGAGCAT CTGGTCGCAT
5701 TGGGTACCA GCAAATCGCG CTGTTAGCGG GCCCATTAAG TTCTGTCTCG GCGCGTCTGC
5761 GTCTGGCTGG CTGGCATAAA TATCTCACTC GCAATCAAAT TCAGCCGATA GCGGAACGGG
5821 AAGGCGACTG GAGTGCCATG TCCGGTTTTT AACAAACCAT GCAAATGCTG AATGAGGGCA
5881 TCGTTCACAC TGCGATGCTG GTTGCCAACG ATCAGATGGC GCTGGGCGCA ATGCGCGCCA
5941 TTACCGAGTC CCGGCTGCGC GTTGGTGCGG ATATCTCGGT AGTGGGATAC GACGATACCG
6001 AAGACAGCTC ATGTTATATC CCGCCGTTAA CCACCATCAA ACAGGATTTT CGCCTGCTGG
6061 GCAAAACCAG CGTGGACCGC TTGCTGCAAC TCTCTCAGGG CCAGGCGGTG AAGGGCAATC
6121 AGCTGTTGCC CGTCTCACTG GTGAAAAGAA AAACCACCCT GGCGCCCAAT ACGCAAACCG-

```

Figure 23C

43/240

6181 CCTCTCCCCG CGCGTTGGCC GATTCATTAA TGCAGCTGGC ACGACAGGTT TCCCGACTGG
6241 AAAGCGGGCA GTGAGCGCAA CGCAATTAAT GTGAGTTAGC TCACTCATTG GGCACCCCAG
6301 GCTTTACACT TTATGCTTCC GGCTCGTATG TTGTGTGGAA TTGTGAGCGG ATAACAATTT
6361 CACACAGGAA ACAGCTATGA CCATGATTAC GGATTCACTG GCCGTCGTTT TACAACGTCG
6421 TGA CTGGGAA AACCTGGCG TTACCCAACT TAATCGCCTT GCAGCACATC CCCCTTTTCGC
6481 CAGCTGGCGT AATAGCGAAG AGGCCCGCAC CGATCGCCCT TCCCAACAGT TGCGCAGCCT
6541 GAATGGCGAA TGGCGCTTTG CCTGGTTTCC GGCACCAGAA GCGGTGCCGG AAAGCTGGCT
6601 GGAGTGCGAT CTTCTGAGG CCGATACTGT CGTCGTCCCC TCAAACGGC AGATGCACGG
6661 TTACGATGCG CCCATCTACA CCAACGTAAC CTATCCCAT TACGGTCAATC CGCCGTTTGT
6721 TCCACGGAG AATCCGACGG GTTGT TACTC GCTCACATT AATGTTGATG AAAGCTGGCT
6781 ACAGGAAGGC CAGACGCGAA TTATTTTGA TGGCGTTGGA ATT

FIGURE 23D

45/240

pDEST4 6964 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
964..1003		Trc
1577..1453		attR1
1827..2486		CmR
2606..2690		inactivated ccdA
2828..3133		ccdB
3174..3298		attR2
3872..4777		ampR
5378..5538		ori
5778..6215		flori (f1 intergenic region)
6587..704		lacIq

1	CTATCCGCTG	GATGACCAGG	ATGCCATTGC	TGTGGAAGCT	GCCTGCACTA	ATGTTCCGGC
61	GTTATTCTT	GATGTCTCTG	ACCAGACACC	CATCAACAGT	ATTATTTTCT	CCCATGAAGA
121	CGGTACGCGA	CTGGGCGTGG	AGCATCTGGT	CGCATTGGGT	CACCAGCAAA	TCGCGCTGTT
181	AGCGGGCCCA	TTAAGTTCTG	TCTCGGCGCG	TCTGCGTCTG	GCTGGCTGGC	ATAAATATCT
241	CACTCGCAAT	CAAATTCAGC	CGATAGCGGA	ACGGGAAGGC	GACTGGAGTG	CCATGTCCGG
301	TTTTCAACAA	ACCATGCAAA	TGCTGAATGA	GGGCATCGTT	CCCACTGCGA	TGCTGGTTGC
361	CAACGATCAG	ATGGCGCTGG	GCGCAATGCG	CGCCATTACC	GAGTCCGGGC	TGCGCGTTGG
421	TGCGGATATC	TCGGTAGTGG	GATACGACGA	TACCGAAGAC	AGCTCATGTT	ATATCCCGCC
481	GTCAACCACC	ATCAAACAGG	ATTTTCGCCT	GCTGGGGCAA	ACCAGCGTGG	ACCGCTTGCT
541	GCAACTCTCT	CAGGGCCAGG	CGGTGAAGGG	CAATCAGCTG	TTGCCCGTCT	CACTGGTGAA
601	AAGAAAAACC	ACCCTGGCAC	CCAATACGCA	AACCGCCTCT	CCCCGCGCGT	TGGCCGATTC
661	ATTAATGCAG	CTGGCACGAC	AGGTTTCCCG	ACTGGAAAGC	GGGCAGTGAG	CGCAACGCAA
721	TTAATGTGAG	TTAGCGCGAA	TTGATCTGGT	TTGACAGCTT	ATCATCGACT	GCACGGTGCA
781	CCAATGCTTC	TGGCGTCAGG	CAGCCATCGG	AAGCTGTGGT	ATGGCTGTGC	AGGTCGTAAA
841	TCACTGCATA	ATTCTGTGTC	CTCAAGGCGC	ACTCCCGTTC	TGGATAATGT	TTTTTGCGCC
901	GACATCATAA	CGGTCTGGC	AAATATTCTG	AAATGAGCTG	TTGACAATTA	ATCATCCGGT
961	CCGTATAATC	TGTGGAATTG	TGAGCGGATA	ACAATTTTAC	ACAGGAAACA	GACCATGGGT
1021	CATCATCATC	ATCATCACGA	TTACGATATC	CCAACGACCG	AAAACCTGTA	TTTTCAGGGC
1081	GCCCATATGA	GCGATAAAAT	TATTCACCTG	ACTGACGACA	GTTTTGACAC	GGATGTACTC
1141	AAAGCGGACG	GGGCGATCCT	CGTCGATTTT	TGGGCAGAGT	GGTGCGGTCC	GTGCAAAATG
1201	ATCGCCCCGA	TTCTGGATGA	AATCGCTGAC	GAATATCAGG	GCAAACTGAC	CGTTGCAAAA
1261	CTGAACATCG	ATCAAAACCC	TGGCACTGCG	CCGAAATATG	GCATCCGTGG	TATCCCGACT
1321	CTGCTGCTGT	TCAAAAACGG	TGAAGTGGCG	GCAACCAAAG	TGGGTGCACT	GTCTAAAGGT
1381	CAGTTGAAAG	AGTTCCTCGA	CGCTAACCTG	GCCGGTTCTG	GTTCTGGTGA	TGACGATGAC
1441	AAGGTACCCA	TCACAAGTTT	GTACAAAAAA	GCTGAACGAG	AAACGTAAAA	TGATATAAAT
1501	ATCAATATAT	TAAATTAGAT	TTTGCAATAA	AAACAGACTA	CATAATACTG	TAAAACACAA
1561	CATATCCAGT	CACTATGGCG	GCCGCTAAGT	TGGCAGCATC	ACCCGACGCA	CTTTGCGCCG
1621	AATAAATACC	TGTGACGGAA	GATCACTTCG	CAGAATAAAT	AAATCCTGGT	GTCCCTGTTG
1681	ATACCGGGAA	GCCCTGGGCC	AACTTTTGGC	GAAAATGAGA	CGTTGATCGA	CACGTAAGAG
1741	GTTCCAACCT	TCACCATAAT	GAAATAAGAT	CACTACCGGG	CGTATTTTTT	GAGTTATCGA
1801	GATTTTTCAGG	AGCTAAGGAA	GCTAAAATGG	AGAAAAAAT	CACTGGATAT	ACCACCGTTG
1861	ATATATCCCA	ATGGCATCGT	AAAGAACATT	TTGAGGCATT	TCAGTCAGTT	GCTCAATGTA
1921	CCTATAACCA	GACCGTTCAG	CTGGATATTA	CGGCCTTTTT	AAAGACCGTA	AAGAAAAATA
1981	AGCACAAAGT	TTATCCGGCC	TTTATTACAA	TTCTTGCCCC	CCTGATGAAT	GCTCATCCGG
2041	AATTCCGTAT	GGCAATGAAA	GACGGTGAGC	TGGTGATATG	GGATAGTGT	CACCTTGTT
2101	ACACCGTTTT	CCATGAGCAA	ACTGAAACGT	TTTCATCGCT	CTGGAGTGAA	TACCACGACG
2161	ATTTCCGGCA	GTTTCTACAC	ATATATTTCG	AAGATGTGGC	GTGTTACGGT	GAAAACCTGG
2221	CCTATTTCCC	TAAAGGGTTT	ATTGAGAATA	TGTTTTTCGT	CTCAGCCAAT	CCCTGGGTGA
2281	GTTTCACCAG	TTTTGATTTA	AACGTGGCCA	ATATGGACAA	CTTCTTCGCC	CCCGTTTTC
2341	CCATGGGCAA	ATATTATACG	CAAGGCGACA	AGGTGCTGAT	GCCGCTGGCG	ATTCAGGTTT
2401	ATCATGCCGT	CTGTGATGGC	TTCCATGTCT	GCAGAATGCT	TAATGAATTA	CAACAGTACT
2461	GCGATGAGTG	GCAGGCGCGG	GCGTAAACGC	GTGGATCCGG	CTTACTAAAA	GCCAGATAAC
2521	AGTATGCGTA	TTTGCGCGCT	GATTTTTTGC	GTATAAGAAT	ATATACTGAT	ATGTATACCC-

FIGURE 24B

46/240

2581 GAAGTATGTC AAAAAGAGGT GTGCTATGAA GCAGCGTATT ACAGTGACAS TTGACAGCGA
 2641 CAGCTATCAG TTGCTCAAGG CATATATGAT GTCAATATCT CCGGTCTGGT AAGCACAACC
 2701 ATGCAGAATG AAGCCCGTCG TCTGCGTGCC GAACGCTGGA AAGCGGAAAA TCAGGAAGGG
 2761 ATGGCTGAGG TCGCCCGGTT TATTGAAATG AACGGCTCTT TTGCTGACGA GAACAGGGAC
 2821 TGGTGAAATG CAGTTTAAAG TTTACACCTA TAAAAGAGAG AGCCGTTATC GTCTGTTTGT
 2881 GGATGTACAG AGTGATATTA TTGACACGCC CGGGCGACGG ATGGTGATCC CCCTGGCCAG
 2941 TGCACGCTCG CTGTCAGATA AAGTCTCCCG TGAACTTTAC CCGGTGGTGC ATATCGGGGA
 3001 TGAAGCTGG CGCATGATGA CCACCGATAT GGCCAGTGTG CCGGTCTCCG TTATCGGGGA
 3061 AGAAGTGGCT GATCTCAGCC ACCGCGAAAA TGACATCAAA AACGCCATTA ACCTGATGTT
 3121 CTGGGGAATA TAAATGTCAG GCTCCCTTAT ACACAGCCAG TCTGCAGGTC GACCATAGTG
 3181 ACTGGATATG TTGTGTTTTA CAGTATTATG TAGTCTGTTT TTTATGCAAA ATCTAATTTA
 3241 ATATATTGAT ATTTATATCA TTTTACGTTT CTCGTTACGC TTTCTTGTA AAAGTGGTGA
 3301 TGGGGATCCT CTAGAGTCGA CCTGCAGTAA TCGTACAGGG TAGTACAAAT AAAAAAGGCA
 3361 CGTCAGATGA CGTGCCTTTT TTCTTGTTGAG CAGTAAGCTT GGCTGTTTTG GCGGATGAGA
 3421 GAAGATTTTC AGCCTGATAC AGATTAAATC AGAACGCAGA AGCGGTCTGA TAAAACAGAA
 3481 TTTGCCTGGC GGCAGTAGCG CGGTGGTCCC ACCTGACCCC ATGCCGAAC CAGAAGTGAA
 3541 ACGCCGTAGC GCCGATGGTA GTGTGGGGTC TCCCCATGCG AGAGTAGGGA ACTGCCAGGC
 3601 ATCAAATAAA ACGAAAGGCT CAGTCGAAAG ACTGGGCCTT TCGTTTTATC TGTTGTTTGT
 3661 CCGTGAACGC TCTCCTGAGT AGGACAAATC CGCCGGGAGC GGATTTGAAC GTTGCGAAGC
 3721 AACGGCCCGG AGGGTGGCGG GCAGGACGCC CGCCATAAAC TGCCAGGCAT CAAATTAAGC
 3781 AGAAGGCCAT CCTGACGGAT GGCCTTTTTG CGTTTCTACA AACTCTTTTT GTTTATTTTT
 3841 CTAAATACAT TCAAAATATGT ATCCGCTCAT GAGACAATAA CCCTGATAAA TGCTTCAATA
 3901 ATATTGAAAA AGGAAGAGTA TGAGTATTCA ACATTTCGT GTCGCCCTTA TTCCCTTTTT
 3961 TGCGGCATTT TGCCTTCCTG TTTTGTCTCA CCCAGAAACG CTGGTGAAAG TAAAAGATGC
 4021 TGAAGATCAG TTGGGTGCAC GAGTGGGTTA CATCGAACTG GATCTCAACA GCGGTAAGAT
 4081 CCTTGAGAGT TTTCCGCCCCG AAGAACGTTT TCCAATGATG AGCACTTTTA AAGTTCTGCT
 4141 ATGTGGCGCG GTATTATCCC GTGTTGACGC CGGGCAAGAG CAACTCGGTC GCCGCATACA
 4201 CTATTCTCAG AATGACTTGG TTGAGTACTC ACCAGTCACA GAAAAGCATC TTACGGATGG
 4261 CATGACAGTA AGAGAATTAT GCAGTGCTGC CATAACCATG AGTGATAACA CTGCGGCCAA
 4321 CTTACTTCTG ACAACGATCG GAGGACCGAA GGAGCTAACC GCTTTTTTGC ACAACATGGG
 4381 GGATCATGTA ACTCGCCTTG ATCGTTGGGA ACCGGAGCTG AATGAAGCCA TACCAAACGA
 4441 CGAGCGTGAC ACCACGATGC CTACAGCAAT GGCAACAACG TTGCGCAAAC TATTAAGTGG
 4501 CGAACTACTT ACTCTAGCTT CCCGGCAACA ATTAATAGAC TGGATGGAGC CGGATAAAGT
 4561 TGCAGGACCA CTTCTGCGCT CGGCCCTTCC GGCTGGCTGG TTTATTGCTG ATAAATCTGG
 4621 AGCCGGTGAG CGTGGGTCTC GCGGTATCAT TGCAGCACTG GGGCCAGATG GTAAGCCCTC
 4681 CCGTATCGTA GTTATCTACA CGACGGGGAG TCAGGCAACT ATGGATGAAC GAAATAGACA
 4741 GATCGCTGAG ATAGGTGCCT CACTGATTAA GCATTGGTAA CTGTCAGACC AAGTTTACTC
 4801 ATATATACTT TAGATTGATT TAAAACCTCA TTTTAAATTT AAAAGGATCT AGGTGAAGAT
 4861 CCTTTTGTAT AATCTCATGA CCAAATCCC TTAACGTGAG TTTTCGTTCC ACTGAGCGTC
 4921 AGACCCCGTA GAAAAGATCA AAGGATCTTC TTGAGATCCT TTTTTTCTGC GCGTAATCTG
 4981 CTGCTTGCAA ACAAAAAAAC CACCGTACC AGCGGTGGTT TGTTTGCCGG ATCAAGAGCT
 5041 ACCAACTCTT TTTCCGAAGG TAACTGGCTT CAGCAGAGCG CAGATACCAA ATACTGTCCT
 5101 TCTAGTGTAG CCGTAGTTAG GCCACCACTT CAAGAACTCT GTAGCACCGC CTACATACCT
 5161 CGCTCTGCTA ATCCTGTTAC CAGTGGCTGC TGCCAGTGGC GATAAGTCGT GTCTTACCGG
 5221 GTTGGACTCA AGACGATAGT TACCGGATAA GGCGCAGCGG TCGGGCTGAA CGGGGGGTTT
 5281 GTGCACACAG CCCAGCTTGG AGCGAACGAC CTACACCGAA CTGAGATACC TACAGCGTGA
 5341 GCTATGAGAA AGCGCCACGC TTCCCGAAGG GAGAAAGGCG GACAGGTATC CGGTAAGCGG
 5401 CAGGGTCGGA ACAGGAGAGC GCACGAGGGA GCTTCCAGGG GGAAACGCCT GGTATCTTTA
 5461 TAGTCCGTGC GGGTTTCGCC ACCTCTGACT TGAGCGTCTGA TTTTGTGTAT GCTCGTCAGG
 5521 GGGGCGGAGC CTATGGAAAA ACGCCAGCAA CGCGGCCTTT TTACGGTTCC TGCCCTTTTG
 5581 CTGGCCTTTT GCTCACATGT TCTTCTCTGC GTTATCCCCT GATTCTGTGG ATAACCGTAT
 5641 TACCGCCTTT GAGTGAGCTG ATACCGCTCG CCGCAGCCGA ACGACCGAGC GCAGCGAGTC
 5701 AGTGAGCGAG GAAGCGGAAG AGCGCCTGAT GCGGTATTTT CTCTTTACGC ATCTGTGCGG
 5761 TATTTACACAC CGCATAATTT TGTTAAAAAT CGCGTTAAAT TTTTGTAAAA TCAGCTCATT
 5821 TTTTAAACCA TAGGCCGAAA TCGGCAAAAT CCCTTATAAA TCAAAAGAAT AGACCGAGAT
 5881 AGGGTTGAGT GTTGTTCAG TTTGGAACAA GAGTCCACTA TTAAAGAAGC TGGACTCCAA
 5941 CGTCAAAGGG CGAAAAACCG TCTATCAGGG CGATGGCCCA CTACGTGAAC CATCACCTTA
 6001 ATCAAGTTTT TTGGGGTCGA GGTGCCGTAA AGCACTAAAT CGGAACCTTA AAGGGAGCCC-

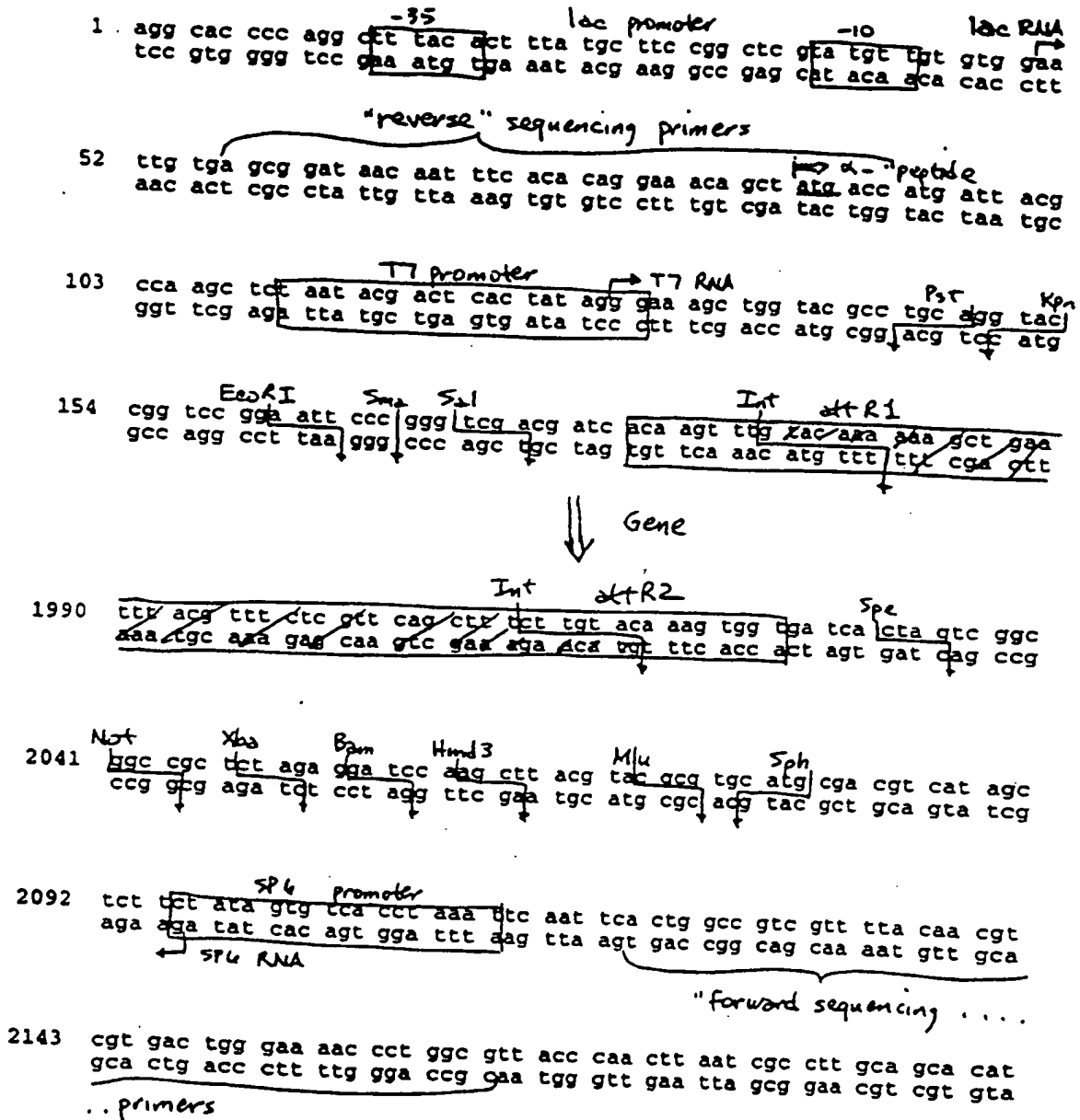
FIGURE 24C

6061 CCGATTTAGA GCTTGACGGG GAAAGCCGGC GAACGTGGCG AGAAAGGAAG GGAAGAAAGC
6121 GAAAGGAGCG GCGCGTAGGG CGCTGGCAAG TG TAGCGGTC ACGCTGCGCG TACCACCAC
6181 ACCCGCCGCG CTTAATGCGC CGCTACAGGG CGCGTCCATT CGCCATTTCAG GCTGCTATGG
6241 TGCACCTCTCA GTACAATCTG CTCTGATGCC GCATAGTTAA GCCAGTATAC ACTCCGCTAT
6301 CGCTACGTGA CTGGGTCATG GCTGCGCCCC GACACCCGCC AACACCCGCT GACGCGCCCT
6361 GACGGGCTTG TCTGCTCCCG GCATCCGCTT ACAGACAAGC TGTGACCGTC TCGGGAGCT
6421 GCATGTGTCA GAGGTTTTCA CCGTCATCAC CGAAACGCGC GAGGCAGCAG ATCAATTTCG
6481 GCGCGAAGGC GAAGCGGCAT GCATTTACGT TGACACCATC GAATGGTGCA AAACCTTTTCG
6541 CGGTATGGCA TGATAGCGCC CGGAAGAGAG TCAATTCAGG GTGGTGAATG TGAAACCAAGT
6601 AACGTTATAC GATGTCGCAG AGTATGCCGG TGTCTCTTAT CAGACCGTTT CCCGCGTGGT
6661 GAACCAGGCC AGCCACGTTT CTGCGAAAAC GCGGGAAAAA GTGGAAGCGG CGATGGCGGA
6721 GCTGAATTAC ATTCCCAACC GCGTGGCACA ACAACTGGCG GGCAAACAGT CGTTGCTGAT
6781 TGGCGTTGCC ACCTCCAGTC TGGCCCTGCA CGCGCCGTCG CAAATTGTCG CGGCGATTAA
6841 ATCTCGCGCC GATCAACTGG GTGCCAGCGT GGTGGTGTCT ATGGTAGAAC GAAGCGGCGT
6901 CGAAGCCTGT AAAGCGGCGG TGCACAATCT TCTCGCGCAA CGCGTCAGTN GGGCTGATCA
6961 TTAA

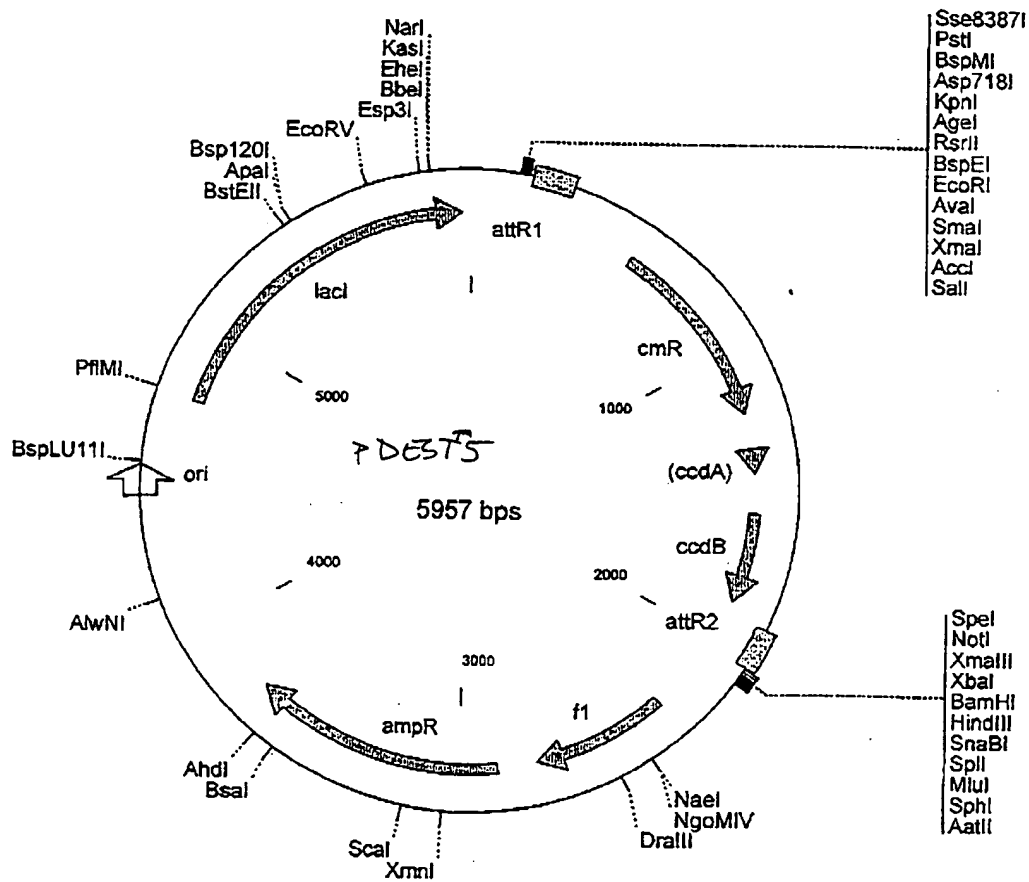
48/240

Figure 25A pDEST5

pSPORT '+' (for sequencing, probes, phagemid)



49/240

Figure 25B γ DEST5 (cont'd)

50/240

pDEST5 5957 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
305..181		attR1
555..1214		CmR
1334..1418		inactivated ccdA
1556..1861		ccdB
1902..2026		attR2
2278..2733		fl (fl intergenic region)
2865..3722		ampR
5378..5538		ori
4756..5922		lacI

1	AGGCACCCCA	GGCTTTACAC	TTTATGCTTC	CGGCTCGTAT	GTTGTGTGGA	ATTGTGAGCG
61	GATAACAATT	TCACACAGGA	AACAGCTATG	ACCATGATTA	CGCCAAGCTC	TAATACGACT
121	CACTATAGGG	AAAGCTGGTA	CGCCTGCAGG	TACCGGTCCG	GAATTCCCGG	GTCGACGATC
181	ACAAGTTTGT	ACAAAAAAGC	TGAACGAGAA	ACGTAAAATG	ATATAAATAT	CAATATATTA
241	AATTAGATTT	TGCATAAAAA	ACAGACTACA	TAATACTGTA	AAACACAACA	TATCCAGTCA
301	CTATGGCGGC	CGCTAAGTTG	GCAGCATCAC	CCGACGCACT	TTGCGCCGAA	TAAATACCTG
361	TGACGGAAGA	TCACTTCGCA	GAATAAATAA	ATCCTGGTGT	CCCTGTTGAT	ACCGGAAGC
421	CCTGGGCCAA	CTTTTGGCGA	AAATGAGACG	TTGATCGGCA	CGTAAGAGGT	TCCAACTTTC
481	ACCATAATGA	AATAAGATCA	CTACCGGGCG	TATTTTGTGA	GTTATCGAGA	TTTTCAGGAG
541	CTAAGGAAGC	TAAATGGAG	AAAAAATCA	CTGGATATAC	CACCGTTGAT	ATATCCCAAT
601	GGCATCGTAA	AGAACATTTT	GAGGCATTTC	AGTCAGTTGC	TCAATGTACC	TATAACCAGA
661	CCGTTCAGCT	GGATATTACG	GCCTTTTAA	AGACCGTAA	GAAAAATAAG	CACAAGTTTT
721	ATCCGGCCTT	TATTCACATT	CTTGCCCGCC	TGATGAATGC	TCATCCGGAA	TTCCGTATGG
781	CAATGAAAGA	CGGTGAGCTG	GTGATATGGG	ATAGTGTTC	CCCTTGTTAC	ACCGTTTTC
841	ATGAGCAAAC	TGAAACGTTT	TCATCGCTCT	GGAGTGAATA	CCACGACGAT	TTCCGGCAGT
901	TTCTACACAT	ATATTCGCAA	GATGTGGCGT	GTTACGGTGA	AAACCTGGCC	TATTTCCCTA
961	AAGGGTTTAT	TGAGAATATG	TTTTTCGTCT	CAGCCAATCC	CTGGGTGAGT	TTCAACAGTT
1021	TTGATTTAAA	CGTGGCCAAT	ATGGACAAC	TCCTCGCCCC	CGTTTTTACC	ATGGGCAAAT
1081	ATTATACGCA	AGGCGACAAG	GTGCTGATGC	CGCTGGCGAT	TCAGGTTTCA	CATGCCGTCT
1141	GTGATGGCTT	CCATGTCCGC	AGAATGCTTA	ATGAATTACA	ACAGTACTGC	GATGAGTGGC
1201	AGGGCGGGCG	GTAACGCGT	GGATCCGGCT	TACTAAAAGC	CAGATAACAG	TATGCGTATT
1261	TGCGCGCTGA	TTTTTGCGGT	ATAAGAATAT	ATACTGATAT	GTATACCCGA	AGTATGTCAA
1321	AAAGAGGTGT	GCTATGAAGC	AGCGTATTAC	AGTGACAGTT	GACAGCGACA	GCTATCAGTT
1381	GCTCAAGGCA	TATATGATGT	CAATATCTCC	GGTCTGGTAA	GCACAACCAT	GCAGAATGAA
1441	GCCCGTCGTC	TGCGTGCCGA	ACGCTGGAAA	GCGGAAAATC	AGGAAGGGAT	GGCTGAGGTC
1501	GCCCGGTTTA	TTGAAATGAA	CGGCTCTTTT	GCTGACGAGA	ACAGGGACTG	GTGAAATGCA
1561	GTTTAAGGTT	TACACCTATA	AAAGAGAGAG	CCGTTATCGT	CTGTTTGTGG	ATGTACAGAG
1621	TGATATTATT	GACACGCCCG	GGCGACGGAT	GGTGATCCCC	CTGGCCAGTG	CACGTCTGCT
1681	GTCAGATAAA	GTCTCCCGTG	AACTTTACCC	GGTGGTGCAT	ATCGGGGATG	AAAGCTGGCG
1741	CATGATGACC	ACCGATATGG	CCAGTGTGCC	GGTCTCCGTT	ATCGGGGAAG	AAGTGGCTGA
1801	TCTCAGCCAC	CGCGAAAATG	ACATCAAAAA	CGCCATTAA	CTGATGTTCT	GGGGAATATA
1861	AATGTCAGGC	TCCCTTATAC	ACAGCCAGTC	TGCAGGTCGA	CCATAGTGAC	TGGATATGTT
1921	GTGTTTTACA	GTATTATGTA	GTCTGTTTTT	TATGCAAAAT	CTAATTTAAT	ATATTGATAT
1981	TTATATCATT	TTACGTTTCT	CGTTCAGCTT	TCTTGTACAA	AGTGGTGATC	ACTAGTCGGC
2041	GGCCGCTCTA	GAGGATCCAA	GCTTACGTAC	CGGTGCATGC	GACGTCATAG	CTCTTCTATA
2101	GTGTCACCTA	AATTCAATTC	ACTGGCCGTC	GTTTTTACAAC	GTCGTGACTG	GGAAAACCTT
2161	GGCGTTACCC	AACTTAATCG	CCTTGCAGCA	CATCCCCCTT	TCGCCAGCTG	GCGTAATAGC
2221	GAAGAGGCC	GCACCGATCG	CCCTTCCCAA	CAGTTGCGCA	GCCTGAATGG	CGAATGGACG
2281	CGCCCTGTAG	CGGCGCATT	AGCGCGGCGG	GTGTGGTGGT	TACGCGCAGC	GTGACCGCTA
2341	CACCTGGCAG	CGCCCTAGCG	CCCGCTCCTT	TCGCTTTCTT	CCCTTCCTTT	CTCGCCACGT
2401	TCGCCCGGCTT	TCCCGTCAA	GCTCTAAATC	GGGGGCTCCC	TTAGGGTTTC	CGATTAGTGT
2461	CTTTACGGCA	CCTCGACCCC	AAAAAACTTG	ATTAGGGTGA	TGGTTACAGT	AGTGGCCAT
2521	CGCCCTGATA	GACGGTTTTT	CGCCCTTTGA	CGTTGGAGTC	CACGTTCTTT	AATAGTGGAC
2581	TCTTGTTCCA	AACTGGAACA	AACTCAACC	CTATCTCGGT	CTATTCTTTT	GATTTATAAG-

FIGURE 25C

51/240

2641 GGATTTTGGCC GATTTCGGCC TATTGGTTAA AAAATGAGCT GATTTAACAA AAATTTAACG
2701 CGAATTTTAA CAAAATATTA ACGTTTACAA TTTTCAGTGG CACTTTTCGG GGAAATGTGC
2761 GCGGAACCCC TATTGTTTTA TTTTCTAAA TACATTCAA TATGTATCCG CTCATGAGAC
2821 AATAACCCCTG ATAAATGCTT CAATAATATT GAAAAAGGAA GAGTATGAGT ATTCACATT
2881 TCCGTGTGCG CTTTATCCC TTTTTGCGG CATTTTGCCT TCCTGTTTTT GCTCACCCAG
2941 AAACGCTGGT GAAAGTAAAA GATGCTGAAG ATCAGTTGGG TGCACGAGTG GGTACATCG
3001 AACTGGATCT CAACAGCGGT AAGATCCTTG AGAGTTTTCG CCCCAGAGAA CGTTTTCCAA
3061 TGATGAGCAC TTTTAAAGTT CTGCTATGCG GCGCGGTATT ATCCCGTATT GACGCCGGG
3121 AAGAGCAACT CGGTCGCCG ATACACTATT CTCAGAATGA CTTGGTTGAG TACTCACCAG
3181 TCACAGAAAA GCATCTTACG GATGGCATGA CAGTAAGAGA ATTATGCAGT GCTGCCATAA
3241 CCATGAGTGA TAACACTGCG GCCAACTTAC TTCTGACAAC GATCGGAGGA CCGAAGGAGC
3301 TAACCGCTTT TTTGCACAAC ATGGGGGATC ATGTAACCTG CTTGATCGT TGGGAACCGG
3361 AGCTGAATGA AGCCATACCA AACGACGAGC GTGACACCAC GATGCCTGTA GCAATGGCAA
3421 CAACGTTGCG CAAACTATTA ACTGGCGAAC TACTTACTCT AGCTTCCCGG CAACAATTAA
3481 TAGACTGGAT GGAGGCGGAT AAAGTTGCAG GACCACTTCT GCGCTCGGCC CTTCCGGCTG
3541 GCTGGTTTTAT TGCTGATAAA TCTGGAGCCG GTGAGCGTGG GTCTCGCGGT ATCATTGCAG
3601 CACTGGGGCC AGATGGTAAG CCTCCCGTA TCGTAGTTAT CTACACGACG GGGAGTCAGG
3661 CAACTATGGA TGAACGAAAT AGACAGATCG CTGAGATAGG TGCCCTCACTG ATTAAGCATT
3721 GGTAACTGTC AGACCAAGTT TACTCATATA TACTTTAGAT TGATTTAAAA CTTCATTTTT
3781 AATTTAAAG GATCTAGGTG AAGATCCTTT TTGATAATCT CATGACCAA ATCCCTTAAC
3841 GTGAGTTTTT GTTCCACTGA GCGTCAGACC CCGTAGAAAA GATCAAAGGA TCTTCTTGAG
3901 ATCCTTTTTT TCTGCGCGTA ATCTGCTGCT TGCAAACAAA AAAACCACCG CTACCAGCGG
3961 TGGTTTGTGTT GCCGGATCAA GAGCTACCAA CTCTTTTTCC GAAGGTAAGT GGCTTCAGCA
4021 GAGCGCAGAT ACCAAATACT GTCCTTCTAG TGTAGCCGTA GTTAGGCCAC CACTTCAAGA
4081 ACTCTGTAGC ACCGCCTACA TACCTCGCTC TGCTAATCCT GTTACCAGTG GCTGCTGCCA
4141 GTGGCGATAA GTCGTGTCTT ACCGGGTTGG ACTCAAGACG ATAGTTACCG GATAAGGCGC
4201 AGCGGTCCGG CTGAACGGGG GGTTCGTGCA CACAGCCCAG CTTGGAGCGA ACGACCTACA
4261 CCGAACTGAG ATACCTACAG CGTGAGCATT GAGAAAGCGC CACGCTTCCC GAAGGGAGAA
4321 AGGCGGACAG GTATCCGGTA AGCGGCAGGG TCGGAACAGG AGAGCGCAGG AGGGAGCTTC
4381 CAGGGGGAAA CGCCTGGTAT CTTTATAGTC CTGTCCGGTT TCGCCACCTC TGACTTGAGC
4441 GTCGATTTTT GTGATGCTCG TCAGGGGGGC GGAGCCTATG GAAAAACGCC AGCAACGCGG
4501 CCTTTTTTACG GTTCTGGGCC TTTTGCTGGC CTTTTGCTCA CATGTTCTTT CCTGCGTTAT
4561 CCCCTGATTC TGTGGATAAC CGTATTACCG CTTTGTAGTG AGCTGATACC GCTCGCCGCA
4621 GCCGAACGAC CGAGCGCAGC GAGTCAGTGA GCGAGGAAGC GGAAGAGCGC CCAATACGCA
4681 AACCGCCTCT CCCCAGCGGT TGGCCGATTC ATTAATGCAG AGCTTGCAAT TCGCGCGCGA
4741 AGGCGAAGCG GCATTACGT TGACACCATC GAATGGCGCA AAACCTTTTCG CGGTATGGCA
4801 TGATAGCGCC CGGAAGAGAG TCAATTCAGG GTGGTGAATG TGAAACCAAGT AACGTTATAC
4861 GATGTCGAG AGTATGCCGG TGTCTCTTAT CAGACCGTTT CCCGCGTGGT GAACCAGGCC
4921 AGCCACGTTT CTGCGAAAAC GCGGGAAAAA GTGGAAGCGG CGATGGCGGA GCTGAATTAC
4981 ATTCCCAACC GCGTGGCACA ACAACTGGCG GGCAACAGT CGTTGCTGAT TGGCGTTGCC
5041 ACCTCCAGTC TGGCCCTGCA CGCGCCGTCG CAAATTGTCG CGGCGATTAA ATCTCGCGCC
5101 GATCAACTGG GTGCCAGCGT GGTGGTGTG ATGGTAGAAC GAAGCGGCGT CGAAGCCTGT
5161 AAAGCGGCGG TGCACAATCT TCTCGCGCAA CGGGTCACTG GGCTGATCAT TAACTATCCG
5221 CTGGATGACC AGGATGCCAT TGCTGTGGAA GCTGCCTGCA CTAATGTTCC GGCCTTATTT
5281 CTTGATGTCT CTGACCAGAC ACCCATCAAC AGTATTATTT TCTCCCATGA AGACGGTACG
5341 CGACTGGGCG TGGAGCATCT GGTGCGATTG GGTCAACAGC AAATCGCGCT GTTAGCGGGC
5401 CCATTAAGTT CTGTCTCGG CCGTCTGCGT CTGGCTGGCT GGCATAAATA TCTCACTCGC
5461 AATCAAATTC AGCCGATAGC GGAACGGGAA GCGGACTGGA GTGCCATGTC CGTTTTTCAA
5521 CAAACCATGC AAATGCTGAA TGAGGGCATC GTTCCCACTG CGATGCTGGT TGCCAACGAT
5581 CAGATGGCGC TGGGCGCAAT GCGCGCCATT ACCGAGTCCG GGCTGCGCGT TGGTGGCGAT
5641 ATCTCGGTAG TGGGATACGA CGATACCGAA GACAGCTCAT GTTATATCCC GCGTCAACC
5701 ACCATCAAAC AGGATTTTCG CCTGCTGGGG CAAACCAGCG TGGACCGCTT GCTGCAACTC
5761 TCTCAGGGCC AGGCGGTGAA GGGCAATCAG CTGTTGCCCG TCTCACTGGT GAAAAGAAAA
5821 ACCACCCTGG CGCCCAATAC GCAAACGCC TCTCCCCGCG CGTTGGCCGA TTCATTAATG
5881 CAGCTGGCAC GACAGTTTC CCGACTGGAA AGCGGGCAGT GAGCGCAACG CAATTAATGT
5941 GAGTTAGCTC ACTCATT

FIGURE 25D

Figure 26A

pDEST6

pSPORT "..."
(opposite strand)

"forward" sequencing primers

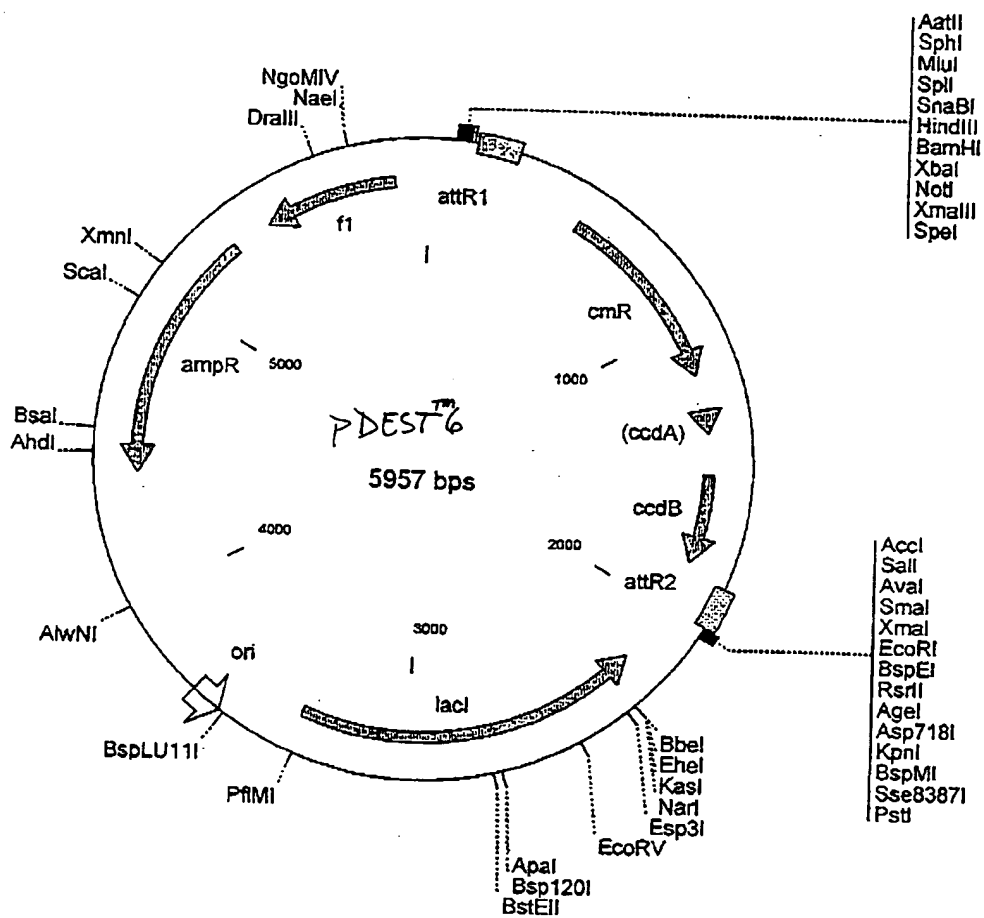
- 1 taa cgc cag ggt ttt ccc agt cac gac gtt gta aaa cga cgg cca gtg aat
att gcg gtc cca aaa ggg tca gtg ctg caa cat ttt gct gcc ggt cac tta
- 52 tga att tag gtg aca cta tag aag agc tat gac gtc gca tgt acg cgt acg
act taa atc cac tgt gat atc ttc tcg ata ctg cag cgt acg tgc gca tgc
- 103 tat gct tgg atc ctc tag agc ggc cgc cga cta gtg atc aca agt tgg taa
att cga acc tag gag atc tcc ccg gcg gct gat cag tag tgt tca aac atg
- 154 aaa daa get gaa cga gaa acg taa aat gat ata aat atc aat ata taa aat
ttt tct cga ctt gct ctt tgc att tta cta tat tca tag tta tat aat tta
- ↓ Gene
- 1939 cat tta tat aat ttt acg ett ctc gtc tag ctt tct tgt aca aag tgg tga
ata aat ata gta aaa tgc aaa gag gaa gtc gaa aga gca tgt ttc acc att
- 1990 tcg tcc acc cgg gaa ttc cgg acc ggt act tgc agg cgt acc agc ttt ccc
agc agc tgg gcc ctt aag gcc tgg cca tgg acg tcc gca tgg tgg aaa ggg
- T7 RNA
- 2041 tat agt gag tgg tat tag agc ttg gcg taa tca tgg tca tag ctg ttt cct
ata tca ctc agc ata atc tgc aac cgc att agt acc agt atc gac aaa gga
- T7 promoter α-peptide "reverse .."
- 2092 gtg tga aat tgt tat ccg ctc aca att cca cac aac ata cga gct gga agc
cac act tta aca ata ggc gag tgt taa ggt gtg ttg tat gct cgg cct tgc
- ... sequencing primers lac RNA
- 2143 ata aag tgt aaa gcc tgg ggt gcc taa tga gtg agc taa ctc aca tta att
tat ttc aca ttt cgg acc cca cgg att act cac tgc att gag tgt aat taa

S3/240

Figure 26B

PDEST6

(cont'd)



54/240

pDEST6 5957 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
266..142		attR1
516..1175		CmR
1295..1379		inactivated ccdA
1517..1822		ccdB
1863..1987		attR2
2203..3369		lacI
4403..5260		ampR
5392..5847		f1 (f1 intergenic region)
1	TAACGCCAGG GTTTTCCCAG TCACGACGTT GTAAAACGAC GGCCAGTGAA TTGAATTTAG	
61	GTGACACTAT AGAAGAGCTA TGACGTCGCA TGCACGCGTA CGTAAGCTTG GATCCTCTAG	
121	AGCGGCCGCC GACTAGTGAT CACAAGTTTG TACAAAAAAG CTGAACGAGA AACGTAAAAT	
181	GATATAAATA TCAATATATT AAATTAGATT TTGCATAAAA AACAGACTAC ATAATACTGT	
241	AAAACACAAC ATATCCAGTC ACTATGGCGG CCGCTAAGTT GGCAGCATCA CCCGACGCAC	
301	TTTGCGCCGA ATAAATACCT GTGACGGAAG ATCACTTCGC AGAATAAATA AATCCTGGTG	
361	TCCCTGTTGA TACCGGGAAG CCCTGGGCCA ACTTTTGGCG AAAATGAGAC GTTGATCGGC	
421	ACGTAAGAGG TTCCAACTTT CACCATAATG AAATAAGATC ACTACCGGGC GTATTTTTTG	
481	AGTTATCGAG ATTTTCAGGA GCTAAGGAAG CTAAAATGGA GAAAAAATC ACTGGATATA	
541	CCACCGTTGA TATATCCCAA TGGCATCGTA AAGAACATTT TGAGGCATTT CAGTCAGTTG	
601	CTCAATGTAC CTATAACCAG ACCGTTGAGC TGGATATTAC GGCTTTTTTA AAGACCGTAA	
661	AGAAAAATAA GCACAAGTTT TATCCGGCCT TTATTCACAT TCTTGCCCGC CTGATGAATG	
721	CTCATCCGGA ATTCCGTATG GCAATGAAAG ACGGTGAGCT GGTGATATGG GATAGTGTTC	
781	ACCTTGTGTA CACCGTTTTT CATGAGCAAA CTGAAACGTT TTCATCGCTC TGGAGTGAAT	
841	ACCACGACGA TTTCCGGCAG TTTCTACACA TATATTGCGA AGATGTGGCG TGTACGGTG	
901	AAAACCTGGC CTATTTCCCT AAAGGGTTTA TTGAGAATAT GTTTTTCGTC TCAGCCAATC	
961	CCTGGGTGAG TTTCAACAGT TTTGATTAA ACGTGGCCAA TATGGACAAC TTCTTCGCCC	
1021	CCGTTTTTAC CATGGGCAAA TATTATACGC AAGGCGACAA GGTGCTGATG CCGCTGGCGA	
1081	TTCAGGTTCA TCATGCCGTC TGTGATGGCT TCCATGTCGG CAGAATGCTT AATGAATTAC	
1141	AACAGTACTG CGATGAGTGG CAGGGCGGGG CGTAAACGCG TGGATCCGGC TTAATAAAG	
1201	CCAGATAACA GTATGCGTAT TTGCGCGCTG ATTTTTGCGG TATAAGAATA TATACTGATA	
1261	TGTATACCCG AAGTATGTCA AAAAGAGGTG TGCTATGAAG CAGCGTATTA CAGTGACAGT	
1321	TGACAGCGAC AGCTATCAGT TGCTCAAGGC ATATATGATG TCAATATCTC CGGTCTGGTA	
1381	AGCACAAACA TGCAGAATGA AGCCCGTCGT CTGCGTGCCG AACGCTGGAA AGCGGAAAAT	
1441	CAGGAAGGGA TGGCTGAGGT CGCCCGTTT ATTGAAATGA ACGGCTCTT TGCTGACGAG	
1501	AACAGGGACT GGTGAAATGC AGTTTAAGGT TTACACCTAT AAAAGAGAGA GCCGTTATCG	
1561	TCTGTTTGTG GATGTACAGA GTGATATTAT TGACACGCCC GGGCGACGGA TGGTGATCCC	
1621	CCTGGCCAGT GCACGTCTGC TGTCAGATAA AGTCTCCCGT GAACTTTACC CGGTGGTGCA	
1681	TATCGGGGAT GAAAGCTGGC GCATGATGAC CACCGATATG GCCAGTGTGC CGGTCTCCGT	
1741	TATCGGGGAA GAAGTGGCTG ATCTCAGCCA CCGCGAAAAT GACATCAAAA ACGCCATTAA	
1801	CCTGATGTTC TGGGGAATAT AAATGTCAGG CTCCCTTATA CACAGCCAGT CTGCAGGTCG	
1861	ACCATAGTGA CTGGATATGT TGTGTTTAC AGTATTATGT AGTCTGTTT TTATGCAAAA	
1921	TCTAATTTAA TATATTGATA TTTATATCAT TTTACGTTTC TCGTTCAGCT TTCTGTACA	
1981	AAGTGGTGAT CGTCGACCCG GGAATTCCGG ACCGGTACCT GCAGGCGTAC CAGCTTTCCC	
2041	TATAGTGAGT CGTATTAGAG CTTGGCGTAA TCATGGTCAT AGCTGTTTCC TGTGTGAAAT	
2101	TGTTATCCGC TCACAATTCC ACACAACATA CGAGCCGGAA GCATAAAGTG TAAAGCCTGG	
2161	GGTGCCTAAT GAGTGAGCTA ACTCACATTA ATTGCGTTGC GCTCACTGCC CGCTTTCCAG	
2221	TCGGGAAACC TGTCGTGCCA GCTGCATTAA TGAATCGGCC AACGCGCGGG GAGAGGCGGT	
2281	TTGCGTATTG GGCGCCAGGG TGGTTTTTCT TTTCAACAGT GAGACGGGCA ACAGCTGATT	
2341	GCCCTTCACC GCCTGGCCCT GAGAGAGTTG CAGCAAGCGG TCCACGCTGG TTTGCCCCAG	
2401	CAGGCGAAAA TCCTGTTTGA TGGTGGTTGA CGGCGGGATA TAACATGAGC TGTCTTCGGT	
2461	ATCGTCGTAT CCCACTACCG AGATATCCGC ACCAACGCGC AGCCCGGACT CGGTAATGGC	
2521	GCGCATTGCG CCCAGCGCCA TCTGATCGTT GGCAACCAGC ATCGCAGTGG GAACGATGCC	
2581	CTCATTGAGC ATTTGTCATG TTTGTTGAAA ACCGGACATG GCACTCCAGT CGCCTTCCCG	
2641	TTCCGCTATC GGCTGAATTT GATTGCGAGT GAGATATTTA TGCCAGCCAG CCAGACGCAG-	

FIGURE 26C

2701 ACGCGCCGAG ACAGAACTTA ATGGGCCCGC TAACAGCGCG ATTTGCTGGT GACCCAATGC
2761 GACCAGATGC TCCACGCCCA GTCGCGTACC GTCCTCATGG GAGAAAATAA TACTGTTGAT
2821 GGGTGTCTGG TCAGAGACAT CAAGAAATAA CGCCGGAACA TTAGTGCAGG CAGCTTCCAC
2881 AGCAATGGCA TCCTGGTCAT CCAGCGGATA GTTAATGATC AGCCCACTGA CCCGTTGCGC
2941 GAGAAGATTG TGCACCGCCG CTTTACAGGC TTCGACGCGC CTTCGTTCTA CCATCGACAC
3001 CACCACGCTG GCACCCAGTT GATCGGCGCG AGATTTAATC GCCGCGACAA TTTGCGACGG
3061 CGCGTGCAGG GCCAGACTGG AGGTGGCAAC GCCAATCAGC AACGACTGTT TGCCCGCCAG
3121 TTGTTGTGCC ACGCGGTTGG GAATGTAATT CAGCTCCGCC ATCGCCGCTT CCACTTTTTT
3181 CCGCGTTTTT GCAGAAACGT GGCTGGCCTG GTTCACCACG CGGGAACGG TCTGATAAGA
3241 GACACCGGCA TACTCTGCGA CATCGTATAA CGTTACTGGT TTCACATCA CCACCTGAA
3301 TTGACTCTCT TCCGGGCGCT ATCATGCCAT ACCCGGAAAG GTTTTGCGCC ATTCGATGGT
3361 GTCAACGTAA ATGCCGCTTC GCCTTCGCGC GCGAATTGCA AGCTCTGCAT TAATGAATCG
3421 CACCAACGCG GGGGAGAGGC GGTTTGCGTA TTGGGCGCTC TTCCGCTTCC TCGTCACTG
3481 ACTCGCTGCG CTCGGTCGTT CGGCTGCCGC GAGCGGTATC AGCTCACTCA AAGCGGTAA
3541 TACGGTTATC CACAGAATCA GGGGATAACG CAGGAAAGAA CATGTGAGCA AAAGGCCAGC
3601 AAAAGGCCAG GAACCGTAAA AAGGCCGCGT TGCTGGCGTT TTTCCATAGG CTCCGCCCCC
3661 CTGACGAGCA TCACAAAAAT CGACGCTCAA GTCAGAGGTG GCGAAACCG ACAGGACTAT
3721 AAAGATACCA GCGGTTTTCC CCTGGAAGCT CCCTCGTGCG CTCTCCTGTT CCGACCCTGC
3781 CGCTTACCGG ATACCTGTCC GCCTTTCTCC CTTGCGGAAG CGTGGCGCTT TCTCAATGCT
3841 CACGCTGTAG GTATCTCAGT TCGGTGTAGG TCGTTCCGTC CAAGCTGGGC TGTGTGCACG
3901 AACCCCCCGT TCAGCCCGAC CGCTGCGCCT TATCCGGTAA CTATCGTCTT GAGTCCAACC
3961 CGGTAAGACA CGACTTATCG CCACTGGCAG CAGCCACTGG TAACAGGATT AGCAGAGCGA
4021 GGTATGTAGG CGGTGCTACA GAGTTCTTGA AGTGTGGGCC TAACTACGGC TACACTAGAA
4081 GGACAGTATT TGGTATCTGC GCTCTGCTGA AGCCAGTTAC CTTGCGAAAA AGAGTTGGTA
4141 GCTCTTGATC CGGCAAAACA ACCACCGCTG GTAGCGGTGG TTTTTTTGTT TGCAAGCAGC
4201 AGATTACGCG CAGAAAAAAA GGATCTCAAG AAGATCCTTT GATCTTTTCT ACGGGGCTG
4261 ACGCTCAGTG GAACGAAAAC TCACGTTAAG GGATTTGGT CATGAGATTA TCAAAAAGGA
4321 TCTTCACCTA GATCCTTTTA AATTAATAAT GAAGTTTAA ATCAATCTAA AGTATATATG
4381 AGTAAACTTG GTCTGACAGT TACCAATGCT TAATCAGTGA GGCACCTATC TCAGCGATCT
4441 GTCTATTTTC TTTCATCCATA GTTGCCCTGAC TCCCCGTCGT GTAGATAACT ACGATACGGG
4501 AGGGCTTACC ATCTGGCCCC AGTGCTGCAA TGATACCGCG AGACCCACGC TCACCGGCTC
4561 CAGATTTATC AGCAATAAAC CAGCCAGCCG GAAGGGCCGA GCGCAGAAGT GGTCTGCAA
4621 CTTTATCCCG CTCCATCCAG TCTATTAATT GTTGCCGGGA AGCTAGAGTA AGTAGTTCCG
4681 CAGTTAATAG TTTGCGCAAC GTTGTTGCCA TTGCTACAGG CATCGTGGTG TCACGCTCGT
4741 CGTTTGGTAT GGCTTCATTC AGCTCCGGTT CCCAACGATC AAGGCGAGTT ACATGATCCC
4801 CCATGTTGTG CAAAAAAGCG GTTAGCTCCT TCGGTCTCTC GATCGTTGTC AGAAGTAAGT
4861 TGGCCGCACT GTTATCACTC ATGGTTATGG CAGCACTGCA TAATTCTCTT ACTGTCATGC
4921 CATCCGTAAG ATGCTTTTCT GTGACTGGTG AGTACTCAAC CAAGTCATTC TGAGAATAGT
4981 GTATGCGGCG ACCGAGTTGC TCTTGCCCGG CGTCAATACG GGATAATACC CGGCCACATA
5041 GCAGAACTTT AAAAGTGCTC ATCATTGGAA AACGTTCTTC GGGGCGAAAA CTCTCAAGGA
5101 TCTTACCGCT GTTGAGATCC AGTTCGATGT AACCCTCTCG TGCACCCAAC TGATCTTCAG
5161 CATCTTTTAC TTTACCAGC GTTTCTGGGT GAGCAAAAAC AGGAAGGCAA AATGCCGCAA
5221 AAAAGGGAAT AAGGGCGACA CGGAAATGTT GAATACTCAT ACTCTTCTTT TTTCAATATT
5281 ATTGAAGCAT TTATCAGGGT TATTGTCTCA TGAGCGGATA CATATTTGAA TGTATTTAGA
5341 AAAATAAACA AATAGGGGTT CCGCGCACAT TTCCCGAAA AGTGCCACCT GAAATTGTAA
5401 ACGTTAATAT TTTGTTAAAA TTCGCGTTAA ATTTTGTGTA AATCAGCTCA TTTTAAACC
5461 AATAGGCCGA AATCGGCAAA ATCCCTTATA AATCAAAAGA ATAGACCGCA ATAGGTTGA
5521 GTGTTGTTCC AGTTTGAAC AAGAGTCCAC TATTAAGAA CGTGGACTCC AACGTCAAAG
5581 GCGGAAAAAC CGTCTATCAG GCGATGGCC CACTACGTGA ACCATACCC TAATCAAGTT
5641 TTTTGGGGTC GAGGTGCCGT AAAGCACTAA ATCGGAACCC TAAAGGGAGC CCCCATTATA
5701 GAGCTTGACG GGGAAAGCCG GCGAACGTGG CGAGAAAGGA AGGGAAGAAA GCGAAAGGAG
5761 CGGGCGCTAG GCGCTGGCA AGTGTAGCGG TCACGCTGCG CGTAACCACC ACACCCGCG
5821 CGCTTAATGC GCGCTACAG GCGCGGTCCA TTCGCTATTC AGGCTGCGCA ACTGTTGGGA
5881 AGGGCGATCG GTGCGGGCCT CTTGCTATT ACGCCAGCTG GCGAAAGGGG GATGTGCTGC
5941 AAGGCGATTA AGTTGGG

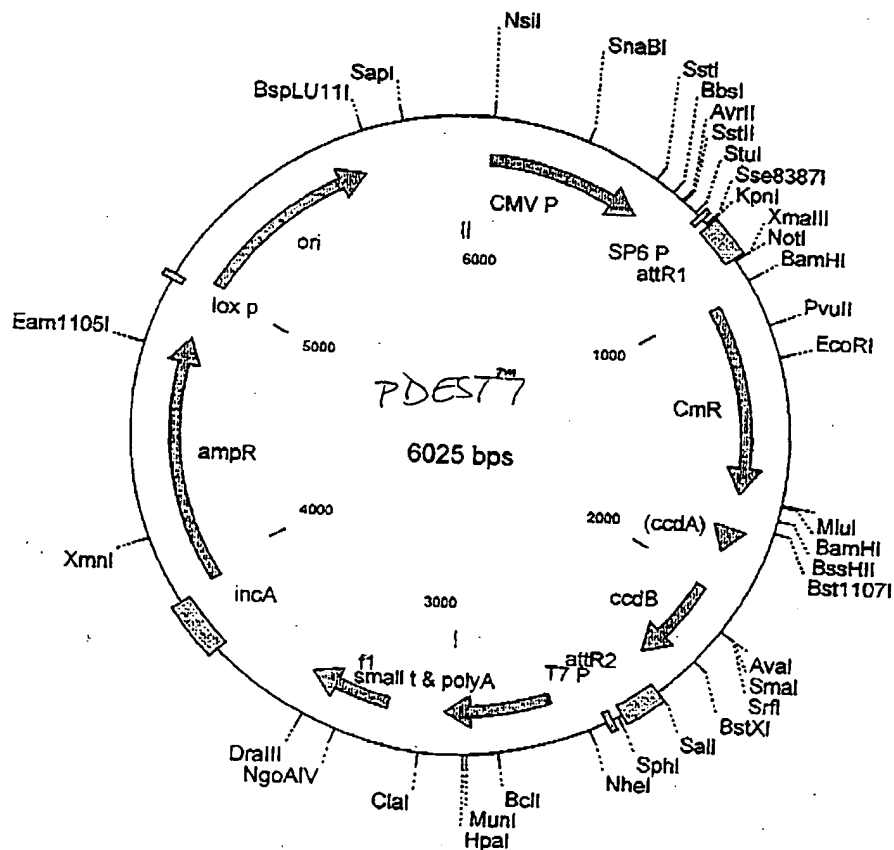
FIGURE 26D

Figure 27A: PDEST7

CMV promoter for eukaryotic expression

970 cca ttg acg caa atg ggc ggt agg cgt gta cgg tgg gag gtc tat ata agc
 ggt aac tgc gtt tac ccg cca tcc gca cat gcc acc ctc cag ata tat tcg
 1021 aga gct cgt tta gtg aac cgt cag atc gcc tgg aga cgc cat cca cgc tgt
 tct cga gca aat cac ttg gca gtc tag cgg acc tct gcg gta ggt gcg aca
 1072 ttt gac ctc cat aga aga cac cgg gac cga tcc agc ctc cgg act cta gcc
 aaa ctg gag gta tct tct gtg gcc ctg gct agg tgg gag gct tga gat cgg
 1123 tag gcc gcg gag cgg ata aca att tca cac agg aaa cag cta tga cca cta
 atc cgg cgc ctc gcc tat tgt taa agt gtg tcc ttt gtc gat act ggt gat
 1174 ggc ttt tgc aaa aag cta ttt agg tga cac tat aga agg tac gcc tgc agg
 ccg aaa acg ttt ttc gat aaa tcc act gtg ata tct tcc atg cgg acg tct
 1225 tac cgg tcc gga att ccc atc aca agt tgg tag aac aag ggt gaa cga gaa
atg gcc agg cct taa ggg tag tgt tca aac atg ttt ttt tga ctc gct ctc

Labels in sequence: *mRNA start* (arrow at 1021), *CMV enhancer/promoter* (bracketed 1072-1123), *Pst* (arrow at 1174), *Kpn* (arrow at 1225), *EcoRI* (arrow at 1225), *attR1* (arrow at 1225).



pDEST7 6025 bp (rotated to position 2800)

<u>Location (Base Nos.)</u>	<u>Gene Encoded</u>
67..589	CMV promoter
906..782	attR1
1015..1674	CmR
1794..1878	inactivated ccdA
2016..2321	ccdB
2362..2486	attR2
2671..3033	small t & polyA
3227..3502	f1
3962..4822	ampR
5022..5661	ori

```

1 ATTATCATGA CATTAACTTA TAAAAATAGG CGTAGTACGA GGCCCTTTCA CTCATTAGAT
61 GCATGTCGTT ACATAACTTA CGGTAAATGG CCCGCCTGGC TGACCGCCCA ACGACCCCCG
121 CCCATTGACG TCAATAATGA CGTATGTTCC CATAGTAACG CCAATAGGGA CTTTCCATTG
181 ACGTCAATGG GTGGAGTATT TACGGTAAAC TGCCCACTTG GCAGTACATC AAGTGTATCA
241 TATGCCAAGT ACGCCCCCTA TTGACGTCAA TGACGGTAA TGGCCCGCCT GGCATTATGC
301 CCAGTACATG ACCTTATGGG ACTTTCCTAC TTGGCAGTAC ATCTACGTAT TAGTCATCGC
361 TATTACCATG GTGATGCGGT TTTGGCAGTA CATCAATGGG CGTGGATAGC GGTTTGACTC
421 ACGGGGATTT CCAAGTCTCC ACCCCATTGA CGTCAATGGG AGTTTGTTTT GGCACCAAAA
481 TCAACGGGAC TTTCCAAAAT GTCGTAACAA CTCCGCCCA TTGACGCAA TGGGCGGTAG
541 GCGTGTACGG TGGGAGGTCT ATATAAGCAG AGCTCGTTTA GTGAACCGTC AGATCGCCTG
601 GAGACGCCAT CCACGCTGTT TTGACCTCCA TAGAAGACAC CGGGACCGAT CCAGCCTCCG
661 GACTCTAGCC TAGGCCGCGG AGCGGATAAC AATTTACAC AGGAAACAGC TATGACCATT
721 AGGCCTTTGC AAAAAGCTAT TTAGGTGACA CTATAGAAGG TACGCCTGCA GGTACCGGAT
781 CACAAGTTTG TACAAAAAAG CTGAACGAGA AACGTAAAT GATATAAATA TCAATATATT
841 AAATTAGATT TTGCATAAAA AACAGACTAC ATAATACTGT AAAACACAAC ATATCCAGTC
901 ACTATGGCGG CCGCATTAGG CACCCAGGC TTTACACTTT ATGCTTCCGG CTCGTATAAT
961 GTGTGGATTT TGAGTTAGGA TCCGTCGAGA TTTTCAGGAG CTAAGGAAGC TAAATGGAG
1021 AAAAAAATCA CTGGATATAC CACCGTTGAT ATATCCCAAT GGCATCGTAA AGAACATTTT
1081 GAGGCATTTC AGTCAGTTGC TCAATGTACC TATAACCAGA CCGTTCAGCT GGATATTACG
1141 GCCTTTTAA AGACCGTAAA GAAAAATAAG CACAAGTTTT ATCCGGCCTT TATTCACATT
1201 CTTGCCCGCC TGATGAATGC TCATCCGGAA TTCCGTATGG CAATGAAAGA CGGTGAGCTG
1261 GTGATATGGG ATAGTGTTCA CCCTTGTTAC ACCGTTTTCC ATGAGCAAAC TGAAACGTTT
1321 TCATCGCTCT GGAGTGAATA CCACGACGAT TTCCGGCAGT TTCTACACAT ATATTGCAA
1381 GATGTGGCGT GTTACGGTGA AAACCTGGCC TATTTCCCTA AAGGGTTTAT TGAGAAATATG
1441 TTTTTCGTCT CAGCCAATCC CTGGGTGAGT TTCACCAATT TTGATTAAAA CGTGGCCAAT
1501 ATGGACAAC TCTTCGCCCC CGTTTTACAC ATGGGCAAAT ATTATACGCA AGGCGACAAG
1561 GTGCTGATGC CGCTGGCGAT TCAGGTTTCAT CATGCCGTCT GTGATGGCTT CCATGTCGGC
1621 AGAATGCTTA ATGAATTACA ACAGTACTGC GATGAGTGGC AGGGCGGGGC GTAAACCGCT
1681 GGATCCGGCT TACTAAAAGC CAGATAACAG TATGCGTATT TGCGCGCTGA TTTTTCGGT
1741 ATAAGAATAT AACTGATAT GTATACCCGA AGTATGTCAA AAAGAGGTGT GCTATGAAGC
1801 AGCGTATTAC AGTGACAGTT GACAGCGACA GCTATCAGTT GCTCAAGGCA TATATGATGT
1861 CAATATCTCC GGTCTGGTAA GCACAACCAT GCAGAATGAA GCCCGTCGTC TGCGTGCCGA
1921 ACGCTGGAAA GCGGAAAATC AGGAAGGGAT GGCTGAGGTC GCCCGGTTTA TTGAAATGAA
1981 CGGCTCTTTT GCTGACGAGA ACAGGGACTG GTGAAATGCA GTTTAAGGTT TACACCTATA
2041 AAAGAGAGAG CCGTTATCGT CTGTTTGTGG ATGTACAGAG TGATATTATT GACACGCCCC
2101 GGCGACGGAT GGTGATCCCC CTGGCCAGTG CACGTCTGCT GTCAGATAAA GTCTCCCGTG
2161 AACTTTACCC GGTGGTGCAT ATCGGGGATG AAAGCTGGCG CATGATGACC ACCGATATGG
2221 CCAGTGTGCC GGTCTCCGTT ATCGGGGAAG AAGTGGCTGA TCTCAGCCAC CGCGAAAAATG
2281 ACATCAAAAA CGCCATTAAC CTGATGTTCT GGGGAATATA AATGTCAGGC TCCCTTATAC
2341 ACAGCCAGTC TGCAGGTGCA CCATAGTGAC TGGATATGTT GTGTTTTTACA GTATTATGTA
2401 GTCTGTTTTT TATGCAAAAT CTAATTTAAT ATATTGATAT TTATATCATT TTACGTTTCT
2461 CGTTCAGCTT TCTGTACAA AGTGGTGATC GCGTGCATGC GACGTCATAG CTCTCTCCCT
2521 ATAGTGAGTC GTATTATAAG CTAGGCACTG GCCGTCGTTT TACAACGTCG TGACTGGGAA-

```

2581 AACTGCTAGC TTGGGATCTT TGTGAAGGAA CCTTACTTCT GTGGTGTGAC ATAAATTGGAC
2641 AAACCTACCTA CAGAGATTTA AAGCTCTAAG GTAAATATAA AATTTTTTAAG TGTATAATGT
2701 GTTAAACTAG CTGCATATGC TTGCTGCTTG AGAGTTTTGC TTAGTGAGTA TGATTTATGA
2761 AAATATTATA CACAGGAGCT AGTGATTCTA ATTGTTTGTG TATTTTAGAT TCACAGTCCC
2821 AAGGCTCATT TCAGGCCCTT CAGTCTCAC AGTCTGTTC AATGATCATAAT CAGCCATACC
2881 ACATTTGTAG AGGTTTTACT TGCTTTAAAA AACCTCCAC ACCTCCCCCT GAACCTGAAA
2941 CATAAAATGA ATGCAATTGT TGTGTTTAAAC TTGTTTATTG CAGCTTATAA TGGTTACAAA
3001 TAAAGCAATA GCATCACAAA TTTCACAAAT AAAGCATTTT TTCACTGCA TTCTAGTTGT
3061 GGTTTGTCCA AACTCATCAA TGTATCTTAT CATGTCTGGA TCGATCCTGC ATTAATGAAT
3121 CGGCCAACGC GCGGGGAGAG GCGGTTTTCG TATTGGCTGG CGTAATAGCG AAGAGGCCCG
3181 CACCGATCGC CCTTCCCAAC AGTTGCGCAG CCTGAATGGC GAATGGGACG CGCCCTGTAG
3241 CGGCGCATT AAGCGCGCGG GTGTGGTGGT TACGCGCAGC GTGACCGCTA CACTTGCCAG
3301 CGCCCTAGCG CCCGCTCCTT TCGCTTTCTT CCTTCCCTT CTGCGCACGT TCGCCGGCTT
3361 TCCCCGTCAA GCTCTAAATC GGGGGCTCCC TTTAGGGTTC CGATTTAGTG CTTTACGGCA
3421 CCTCGACCCC AAAAACTTG ATTAGGGTGA TGGTTCACGT AGTGGGCCAT CGCCCTGATA
3481 GACGGTTTTT CGCCCTTGA CGTTGGAGTC CACGTTCTTT AATAGTGGAC TCTTGTCCA
3541 AACTGGAACA AACTCAACC CTATCTCGGT CTATCTTTT GATTTATAAG GGATTTTGCC
3601 GATTTTCGCC TATTGGTTAA AAAATGAGCT GATTTAACAA AAATTTAACG CGAATTTTAA
3661 CAAAATATTA ACGTTTACAA TTTCAGGTGG CACTTTTCGG GGAAATGTGC GCGGAACCCC
3721 TATTTGTGTTA TTTTCTTAAA TACATTCAAA TATGTATCCG CTCATGCCAG GTCTTGGACT
3781 GGTGAGAACG GCTTGTCTCG CAGCTTCGAT GTGTGCTGGA GGGAGAATAA AGGTCTAAGA
3841 TGTGCGATAG AGGGAAGTCG CATTGAATTA TGTGCTGTGT AGGGATCGCT GGTATCAAAT
3901 ATGTGTGCCC ACCCCTGGCA TGAGACAATA ACCCTGATAA ATGCTTCAAT AATATTGAAA
3961 AAGGAAGAGT ATGAGTATTC AACATTTCCG TGTGCGCCTT ATTCCCTTTT TTGCGGCATT
4021 TTGCCTTCCT GTTTTTGCTC ACCCAGAAAC GCTGGTGAAA GTAAAAGATG CTGAAGATCA
4081 GTTGGGTGCA CGAGTGGGTT ACATCGAACT GGATCTCAAC AGCGGTAAGA TCCTTGAGAG
4141 TTTTCGCCCC GAAGAACGTT TTCCAATGAT GAGCACTTTT AAAGTTCCTG TATGTGGCGC
4201 GGTATTATCC CGTATTGACG CCGGGCAAGA GCAACTCGGT CGCCGCATAC ACTATTCTCA
4261 GAATGACTTG GTTGAGTACT CACCAGTCAC AGAAAAGCAT CTTACGGATG GCATGACAGT
4321 AAGAGAATTA TGCAGTGCTG CCATAACCAT GAGTGATAAC ACTGCGGCCA ACTTACTTCT
4381 GACAACGATC GGAGGACCGA AGGAGCTAAC CGCTTTTTTG CACAACATGG GGGATCATGT
4441 AACTCGCCTT GATCGTTGGG AACCGGAGCT GAATGAAGCC ATACCAAACG ACGAGCGTGA
4501 CACCAAGATG CCTGTAGCAA TGGCAACAAC GTTGGCAGAA CTATTAACATG GCGAACTACT
4561 TACTCTAGCT TCCCGCAAC AATTAATAGA CTGGATGGAG GCGGATAAAG TTGCAAGGAC
4621 ACTTCTGCGC TCGGCCCTTC CGGCTGGCTG GTTTATTGCT GATAAATCTG GAGCCGGTGA
4681 GCGTGGGTCT CGCGGTATCA TTGCAGCACT GGGGCCAGAT GGTAAGCCCT CCCGTATCGT
4741 AGTTATCTAC ACGACGGGGA GTCAGGCAAC TATGGATGAA CGAAATAGAC AGATCGCTGA
4801 GATAGGTGCC TCACTGATTA AGCATTGGTA ACTGTCAGAC CAAGTTTACT CATATATACT
4861 TTAGATTGAT TTAAAACTTC ATTTTAAAT TAAAGGATC TAGGTGAAGA TCCTTTTGA
4921 TAATCTCATG CCATAACTTC GTATAATGTA TGCTATACGA AGTTATGGCA TGACCAAAAT
4981 CCCTTAACGT GAGTTTTCTG TCCACTGAGC GTCAGACCCC GTAGAAAAGA TCAAAGGATC
5041 TTCTTGAGAT CCTTTTTTTC TGCGCGTAAT CTGCTGCTTG CAAACAAAAA AACCACCGCT
5101 ACCAGCGGTG GTTTGTTTGC CGGATCAAGA GCTACCAACT CTTTTTCCGA AGGTAAGTGG
5161 CTTACAGACA GCGCAGATAC CAAATACTGT CCTTCTAGTG TAGCCGTAGT TAGGCCACCA
5221 CTTCAAGAAC TCTGTAGCAC CGCCTACATA CCTCGCTCTG CTAATCCTGT TACCAGTGGC
5281 TGCTGCCAGT GCGGATAAGT CGTGCTTTAC CGGTTTGGAC TCAAGACGAT AGTTACCGGA
5341 TAAGGCGCAG CGGTGCGGCT GAACGGGGGG TTCGTGCACA CAGCCCAGCT TGGAGCGAAC
5401 GACCTACACC GAACTGAGAT ACCTACAGCG TGAGCATTGA GAAAGCGCCA CGCTTCCCGA
5461 AGGGAGAAAAG GCGGACAGGT ATCCGGTAAG CGGCAGGGTC GGAACAGGAG AGCGCACGAG
5521 GGAGCTTCCA GGGGGAAAACG CCTGGTATCT TTATAGTCCT GTCGGGTTTC GCCACCTCTG
5581 ACTTGAGCGT CGATTTTTGT GATGCTCGTC AGGGGGGCGG AGCCTATGGA AAAACGCCAG
5641 CAACGCGGCC TTTTACGGT TCCTGGCCTT TTGCTGGCCT TTTGCTCACA TGTTCTTTCC
5701 TGCGTTATCC CCTGATTCTG TGGATAACCG TATTACCGCC TTTGAGTGAG CTGATACCGC
5761 TCGCCGACG CGAACGACCG AGCGCAGCGA GTCAGTGAGC GAGGAAGCGG AAGAGCGCCC
5821 AATACGCAAA CCGCCTCTCC CCGCGGCTTG GCCGATTCAT TAATGCAGAG CTTGCAATTC
5881 GCGCGTTTTT CAATATTATT GAAGCATTTA TCAGGGTTAT TGTCTCATGA GCGGATACAT
5941 ATTTGAATGT ATTTAGAAAA ATAAACAAAT AGGGGTTCCG CGCACATTC CCCGAAAAGT
6001 GCCACCTGAC GTCTAAGAAA CCATT

Figure 28A: pDEST8 Polyhedron Promoter, Baculovirus Transfer Plasmid

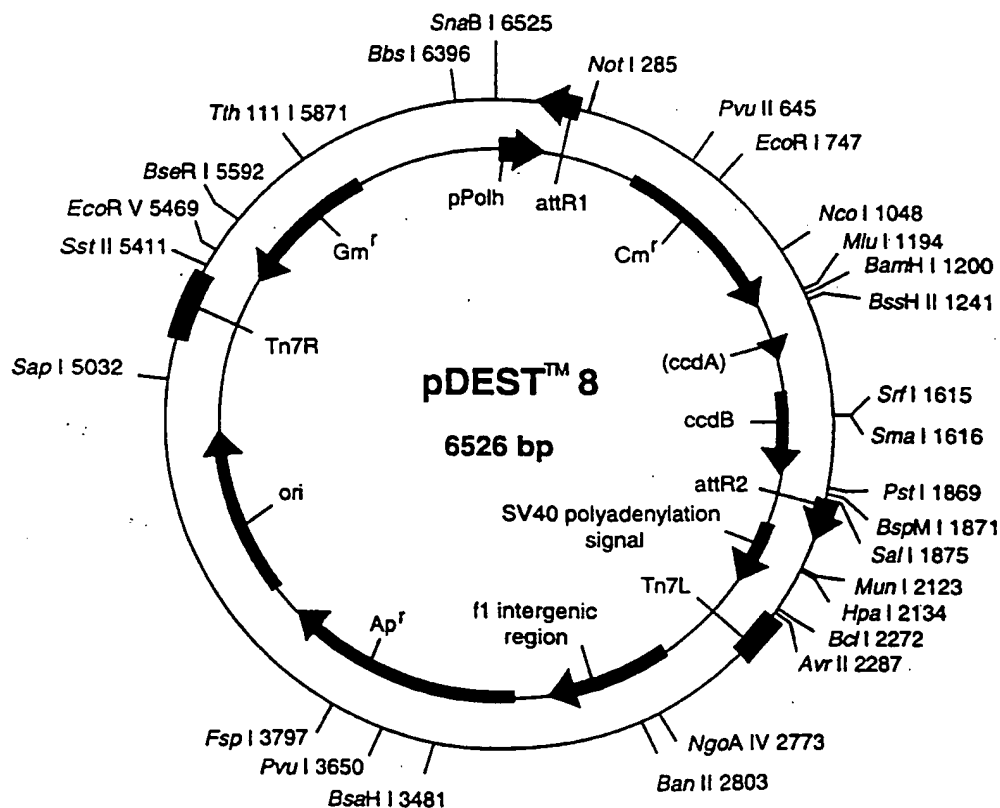
Ace I

```

1  cgt|ata ctc cgg aat att aat aga tca tgg aga taa tta aaa tga taa cca
   gca tat gag gcc tta taa tta tct agt acc tct att aat ttt act att ggt
52  tct cgc aaa taa ata agt att tta ctg ttt tgg taa cag ttt tgt aat aaa
   aga gcg ttt att tat tca taa aat gac aaa agc att gtc aaa aca tta ttt
103 aaa acc tat aaa tat tcc gga tta ttc ata cgg tcc cac cat cgg gcg cgg
   ttt tgg ata ttt ata agg cct aat aag tat ggc agg gtg gta gcc cgc gcc
154 atc atc aca agt tgg tgg aca aca gct gaa cga gaa aag taa aat aat ata
   tag tag tgt tca aac atg ttt ttt cga ctt gct ctt tgc att tta cta tat

```

Handwritten notes:
 - Arrow pointing to position 1: **Ace I**
 - Arrow pointing to position 52: **mRNA (polyhedrin)**
 - Arrow pointing to position 154: **Pam**
 - Arrow pointing to position 154: **Start**
 - Arrow pointing to position 154: **attR1**



60/240

pDEST8 6526 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
23..152		Ppolh
284..160		attR1
534..1193		CmR
1313..1397		inactivated ccdA
1535..1840		ccdB
1881..2005		attR2
2766..3146		f1
3240..4090		ampR
4289..4869		ori
5564..6496		genR

1	CGTATACTCC	GGAATATTAA	TAGATCATGG	AGATAATTAA	AATGATAACC	ATCTCGCAAA
61	TAAATAAGTA	TTTTACTGTT	TTCGTAACAG	TTTTGTAATA	AAAAAACCTA	TAAATATTCC
121	GGATTATTCA	TACCGTCCCA	CCATCGGGCG	CGGATCATCA	CAAGTTTGTA	CAAAAAAGCT
181	GAACGAGAAA	CGTAAAATGA	TATAAATATC	AATATATTAA	ATTAGATTTT	GCATAAAAAA
241	CAGACTACAT	AATACTGTAA	AACACAACAT	ATCCAGTCAC	TATGGCGGCC	GCTAAGTTGG
301	CAGCATCACC	CGACGCACCT	TGCGCCGAAT	AAATACCTGT	GACGGAAGAT	CACTTCGCAG
361	AATAAATAAA	TCCTGGTGTC	CCTGTTGATA	CCGGGAAGCC	CTGGGCCAAC	TTTGGCGAA
421	AATGAGACGT	TGATCGGCAC	GTAAGAGGTT	CCAACTTTCA	CCATAATGAA	ATAAGATCAC
481	TACCGGGCGT	ATTTTTTGAG	TTATCGAGAT	TTTCAGGAGC	TAAGGAAGCT	AAAATGGAGA
541	AAAAAATCAC	TGGATATACC	ACCGTTGATA	TATCCCAATG	GCATCGTAAA	GAACATTTTG
601	AGGCATTTCA	GTGAGTTGCT	CAATGTACCT	ATAACCAGAC	CGTTCAGCTG	GATATTACGG
661	CCTTTTTAAA	GACCGTAAAG	AAAAATAAGC	ACAAGTTTTA	TCCGGCCTTT	ATTACATTTC
721	TTGCCCGCCT	GATGAATGCT	CATCCGGAAT	TCCGTATGGC	AATGAAAGAC	GGTGAGCTGG
781	TGATATGGGA	TAGTGTTTCA	CCTTGTACAC	CCGTTTTCCA	TGAGCAAAC	GAAACGTTTT
841	CATCGCTCTG	GAGTGAATAC	CACGACGATT	TCCGGCAGTT	TCTACACATA	TATTCGCAAG
901	ATGTGGCGTG	TTACGGTGAA	AACCTGGCCT	ATTTCCCTAA	AGGGTTTATT	GAGAATATGT
961	TTTTCTCTC	AGCCAATCCC	TGGGTGAGTT	TCACCAGTTT	TGATTTAAAC	GTGCCCAATA
1021	TGGACAACCT	CTTCGCCCCC	GTTTTTACCA	TGGGCAAATA	TTATACGCAA	GGCGACAAGG
1081	TGCTGATGCC	GCTGGCGATT	CAGGTTTCATC	ATGCCGTCCTG	TGATGGCTTC	CATGTCGGCA
1141	GAATGCTTAA	TGAATTACAA	CAGTACTGCG	ATGAGTGGCA	GGGCGGGGCG	TAAACGCGTG
1201	GATCCGGCTT	ACTAAAAGCC	AGATAACAGT	ATGCGTATTT	GCGCGCTGAT	TTTTGCGGTA
1261	TAAGAATATA	TACTGATATG	TATACCCGAA	GTATGTCAAA	AAGAGGTGTG	CTATGAAGCA
1321	GCGTATTACA	GTGACAGTTG	ACAGCGACAG	CTATCAGTTG	CTCAAGGCAT	ATATGATGTC
1381	AATATCTCCG	GTCTGGTAAG	CACAACCATG	CAGAATGAAG	CCCGTCGTCT	GCGTGCCGAA
1441	CGCTGGAAAG	CGGAAAATCA	GGAAGGGATG	GCTGAGGTG	CCCGGTTTAT	TGAAATGAAC
1501	GGCTCTTTTG	CTGACGAGAA	CAGGGACTGG	TGAAATGCAG	TTTAAGGTTT	ACACCTATAA
1561	AAGAGAGAGC	CGTTATCGTC	TGTTTGTGGA	TGTACAGAGT	GATATTATG	ACACGCCCGG
1621	GCGACGGATG	GTGATCCCCC	TGGCCAGTGC	ACGTCTGCTG	TCAGATAAAG	TCTCCCGTGA
1681	ACTTTACCCG	GTGGTGCCATA	TCGGGGATGA	AAGCTGGCGC	ATGATGACCA	CCGATATGGC
1741	CAGTGTGCCG	GTCTCCGTTA	TCGGGGAAGA	AGTGGCTGAT	CTCAGCCACC	GCGAAAATGA
1801	CATCAAAAAC	GCCATTAACC	TGATGTTCTG	GGGAATATAA	ATGTCAGGCT	CCCTTATACA
1861	CAGCCAGTCT	GCAGGTCGAC	CATAGTGACT	GGATATGTTG	TGTTTTACAG	TATTATGTAG
1921	TCTGTTTTTT	ATGCAAAATC	TAATTTAATA	TATTGATATT	TATATCATTT	TACGTTTCTC
1981	GTTTCAGCTTT	CTTGTAACAA	GTGGTGATAG	CTTGTCGAGA	AGTACTAGAG	GATCATAATC
2041	AGCCATACCA	CATTTGTAGA	GGTTTTACTT	GCTTTAAAAA	ACCTCCCA	CCTCCCCCTG
2101	AACCTGAAAC	ATAAAATGAA	TGCAATTGTT	GTTGTTAACT	TGTTTATGTC	AGCTTATAAT
2161	GGTTACAAAT	AAAGCAATAG	CATCACAAT	TTCAACAAATA	AAGCATTTTT	TTCACTGCAT
2221	TCTAGTTGTG	GTTTGTCCAA	ACTCATCAAT	GTATCTTATC	ATGCTGGATG	CTGATCACTG
2281	CTTGAGCCTA	GGAGATCCGA	ACCAGATAAG	TGAAATCTAG	TTCCAACTA	TTTTGTCTAT
2341	TTTAATTTTC	GTATTAGCTT	ACGACGCTAC	ACCCAGTTCC	CATCTATTTT	GTCACTCTTC
2401	CCTAAATAAT	CCTTAAAAAC	TCCATTTCCA	CCCCCTCCAG	TTCCCAACTA	TTTTGTCCGC
2461	CCACAGCGGG	GCATTTTCT	TCCTGTTATG	TTTTTAATCA	AACATCCTGC	CAACTCCATG
2521	TGACAAACCG	TCATCTTCGG	CTACTTTTTC	TCTGTACAG	AATGAAAATT	TTTCTGTCAT

FIGURE 28B

2581 CTCTTCGTTA TTAATGTTTG TAATTGACTG AATATCAACG CTTATTTGCA GCCTGAATGG
2641 CGAATGGACG CGCCCTGTAG CGGCGCATT AAGCGGCGG GTGTGGTGGT TACGCGCAGC
2701 GTGACCGCTA CACTTGCCAG CGCCCTAGCG CCCGCTCCTT TCGCTTTCTT CCCTTCCTTT
2761 CTCGCCACGT TCGCCGGCTT TCCCCGTCAA GCTCTAAATC GGGGGCTCCC TTTAGGGTTC
2821 CGATTTAGTG CTTTACGGCA CCTCGACCCC AAAAACTTG ATTAGGGTGA TGGTTCACGT
2881 AGTGGGCCAT CGCCCTGATA GACGGTTTTT CGCCCTTTGA CGTTGGAGTC CACGTTCTTT
2941 AATAGTGGAC TCTTGTTCCA AACTGGAACA AACTCAACC CTATCTCGGT CTATTCTTTT
3001 GATTTATAAG GGATTTTGCC GATTTTCGGC TATTGGTTAA AAAATGAGCT GATTAACAA
3061 AAATTTAACG CGAATTTTAA CAAAATATTA ACGTTTACAA TTTTCAGGTG CACTTTTCGG
3121 GGAAATGTGC GCGGAACCCC TATTTGTTTA TTTTCTTAA TACATTCAA TATGTATCCG
3181 CTCATGAGAC AATAACCTG ATAAATGCTT CAATAATATT GAAAAAGGAA GAGTATGAGT
3241 ATTCAACATT TCCGTGTCGC CTTATTCCC TTTTTCGCG CATTTTGCCT TCCTGTTTTT
3301 GCTCACCCAG AAACGCTGGT GAAAGTAAA GATGCTGAAG ATCAGTTGGG TGCACGAGTG
3361 GGTTACATCG AACTGGATCT CAACAGCGGT AAGATCCTTG AGAGTTTTTCG CCCCAGAGAA
3421 CGTTTTCCAA TGATGAGCAC TTTTAAAGTT CTGCTATGTG GCGCGGTATT ATCCCGTATT
3481 GACGCCGGGC AAGAGCAACT CGGTCGCCGC ATACACTATT CTCAGAATGA CTTGGTTGAG
3541 TACTCACCG TACAGAAAA GCATCTTACG GATGGCATGA CAGTAAGAGA ATTATGCAGT
3601 GCTGCCATAA CCATGAGTGA TAACACTGCG GCCAACTTAC TTCTGACAAC GATCGGAGGA
3661 CCGAAGGAGC TAACCGCTTT TTGACACAAC ATGGGGGATC ATGTAACCTG CTTGATCGT
3721 TGGGAACCGG AGCTGAATGA AGCCATACCA AACGACGAGC GTGACACCAC GATGCCGTGA
3781 GCAATGGCAA CAACGTTGCG CAACTATTA ACTGGCGAAC TACTTACTCT AGCTTCCCGG
3841 CAACAATTAA TAGACTGGAT GGAGGCGGAT AAAGTTGCAG GACCACTTCT GCGCTCGGCC
3901 CTTCCGGCTG GCTGGTTTAT TGCTGATAAA TCTGGAGCCG GTGAGCGTGG GTCTCGCGGT
3961 ATCATTGCAG CACTGGGGCC AGATGGTAAG CCCTCCCGTA TCGTAGTTAT CTACACGAGC
4021 GGGAGTCAGG CAACTATGGA TGAACGAAAT AGACAGATCG CTGAGATAGG TGCCTCACTG
4081 ATTAAGCATT GGTAACGTG AGACCAAGTT TACTCATATA TACTTTAGAT TGATTTAAAA
4141 CTTCAATTTT AATTTAAAG GATCTAGGTG AAGATCCTTT TTGATAATCT CATGACCAA
4201 ATCCCTTAAC GTGAGTTTTT GTTCCACTGA GCGTCAGACC CCGTAGAAAA GATCAAAGGA
4261 TCTTCTTGAG ATCCTTTTTT TCTGCGCGTA ATCTGCTGCT TGCAAACAAA AAAACCACCG
4321 CTACACGCGG TGGTTTGTG GCGGATCAA GAGCTACCAA CTCTTTTTCC GAAGGTAAC
4381 GGCTTCAGCA GAGCGCAGAT ACCAAATACT GTCCTTCTAG TGTAGCCGTA GTTAGGCCAC
4441 CACTTCAAGA ACTCTGTAGC ACCGCTACA TACCTCGCTC TGCTAATCCT GTTACCAGTG
4501 GCTGTGCCA GTGGCGATAA GTCGTGTCTT ACCGGGTTGG ACTCAAGACG ATAGTTACCG
4561 GATAAGGCGC AGCGGTCGGG CTGAACGGGG GGTTCGTGCA CACAGCCAG CTTGGAGCGA
4621 ACGACCTACA CCGAACTGAG ATACCTACAG CGTGAGCATT GAGAAAGCGC CACGCTTCCC
4681 GAAGGGAGAA AGGCGGACAG GTATCCGGTA AGCGGCAGGG TCGGAACAGG AGAGCGCACG
4741 AGGGAGCTTC CAGGGGGAAG CGCCTGGTAT CTTTATAGTC CTGTGCGGTT TCGCCACCTC
4801 TGACTTGAGC GTCGATTTTT GTGATGCTCG TCAGGGGGGC GGAGCCATAG GAAAAACGCC
4861 AGCAACGCGG CCTTTTACG GTTCCCTGCC TTTTGCTGGC CTTTGTCTCA CATGTTCTTT
4921 CCTGCGTTAT CCCCTGATTC TGTGGATAAC CGTATTACCG CCTTTGAGTG AGCTGATACC
4981 GCTCGCCGCA GCCGAACGAC CGAGCGCAGC GAGTCAGTGA GCGAGGAAGC GGAAGAGCGC
5041 CTGATGCGGT ATTTTCTCCT TACGCATCTG TCGGTATTT CACACCGCAG ACCAGCCGCG
5101 TAACCTGGCA AAATCGGTTA CGGTTGAGTA ATAAATGGAT GCCCTGCGTA AGCGGGTGTG
5161 GCGGACAAT AAAGTCTTAA ACTGAACAAA ATAGATCTAA ACTATGACAA TAAAGTCTTA
5221 AACTAGACAG AATAGTTGTA AACTGAAATC AGTCCAGTTA TGCTGTGAAA AAGCATACTG
5281 GACTTTTGTG ATGGCTAAAG CAAACTCTTC ATTTTCTGAA GTGCAAATTG CCCGTCGTAT
5341 TAAAGAGGGG CGTGGCCAAG GGCATGGTAA AGACTATATT CGCGGCGTTG TGACAAATTA
5401 CCGAACAACT CCGCGGCCGG GAAGCCGATC TCGGCTTGAA CGAATTGTTA GGTGGCGGTA
5461 CTTGGGTCGA TATCAAAGTG CATCACTTCT TCCCGTATGC CCAACTTTGT ATAGAGAGCC
5521 ACTGCGGGAT CGTCACCGTA ATCTGCTTGC ACGTAGATCA CATAAGCACC AAGCGCGTTG
5581 GCCTCATGCT TGAGGAGATT GATGAGCGCG GTGGCAATGC CCTGCCTCCG GTGCTCGCCG
5641 GAGACTGCGA GATCATAGAT ATAGATCTCA CTACGCGGCT GCTCAAACCT GGGCAGAACG
5701 TAAGCCGCGA GAGCGCCAAC AACCGCTTCT TGGTCGAAGG CAGCAAGCGC GATGAATGTC
5761 TTAAGTACGGA GCAAGTTCCC GAGGTAATCG GAGTCCGCT GATGTTGGGA GTAGGTGGCT
5821 ACGTCTCCGA ACTCACGACC GAAAAGATCA AGAGCAGCCC GCATGGATTT GACTTGGTCA
5881 GGCCGAGCC TACATGTGCG AATGATGCCC ATACTTGAGC CACCTAACTT TGTTTTAGGG
5941 CGACTGCCCT GTCGCGTAAC ATCGTTGCTG CTGCGTAACA TCGTTGCTGC TCCATAACAT
6001 CAAACATCGA CCCACGGCGT AACCGCTTG CTGCTTGAT GCCCGAGGCA TAGACTGTAC-

62/240

6061 AAAAAACAG TCATAACAAG CCATGAAAAC CGCCACTGCG CCGTTACCAC CGCTGCGTTC
6121 GGTCAAGGTT CTGGACCAGT TCGTGAGCG CATACGCTAC TTGCATTACA GTTTACGAAC
6181 CGAACAGGCT TATGTCAACT GGGTTCGTGC CTTTCATCCGT TTCCACGGTG TCGGTCACCC
6241 GGCAACCTTG GGCAGCAGCG AAGTCGAGGC ATTTCTGTCC TGGCTGGCGA ACGAGCGCAA
6301 GGTTTCGGTC TCCACGCATC GTCAGGCATT GGCGGCCTTG CTGTTCTTCT ACGGCAAGGT
6361 GCTGTGCACG GATCTGCCCT GGCTTCAGGA GATCGGAAGA CCTCGGCCGT CGCGGCGCTT
6421 GCCGGTGGTG CTGACCCCGG ATGAAGTGGT TCGCATCCTC GGTTTTCTGG AAGGCGAGCA
6481 TCGTTTGTTT GCCCAGGACT CTAGCTATAG TTCTAGTGGT TGGCTA

FIGURE 28D

$$6^3/240$$

Figure 29A: PDCT9

Semliki Forest Virus vector

103 ttg gcg agg gac att aag gcg ttt aag aaa ttg aga gga cct gtt ata gac
aac cgc tcc ctg taa ttc cgc aaa ttc ttt aac tct cct gga caa tat gtg

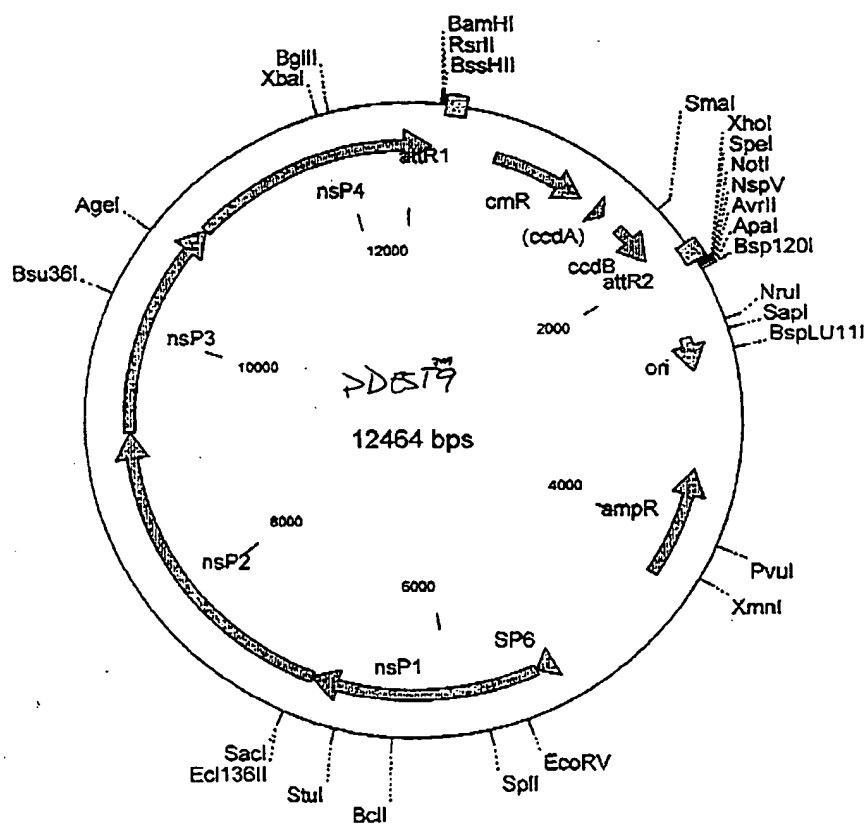
243 promoter → 245 RNA

154 ctc tac ggc ggt cct aga ttg gtg cgt taa tac aca gaa ttc tga ttg gat
gag atg ccg cca gga tct aac cac gca att atg tgt ctt aag act aac cta

Rsr II

205 ccc ggt ccg aag cgc gct ttc cca tca aca agt ttg tac aac aag gct gaa
ggg cca ggc ttc gcg cga aag ggt agt tgt tca aac atg ttt ttc cga ttc

Int att R1



64/240

pDEST9 12464 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
355..232		attR1
605..1264		CmR
1384..1468		inactivated ccdA
1606..1911		ccdB
1952..2078		attR2
2532..2782		ori
3482..4282		ampR
5232..5365		SP6 promoter
5365..6965		nsP1:non-structural protein 1
6965..9265		nsP2:non-structural protein 2
9265..10865		nsP3:non-structural protein 3
10865..161		nsP4:non-structural protein 4
1	AGCAAGTGGT TCCGGACAGG CTTGGGGGCC GAAGTGGAGG TGGCACTAAC ATCTAGGTAT	
61	GAGGTAGAGG GCTGCAAAAG TATCCTCATA GCCATGGCCA CCTTGGCGAG GGACATTAAG	
121	GCGTTTAAGA AATTGAGAGG ACCTGTTATA CACCTCTACG GCGGTCCTAG ATTGGTGCCT	
181	TAATACACAG AATTCTGATT GGATCCCGGT CCGAAGCGCG CTTTCCCATC ACAAGTTTGT	
241	ACAAAAAAGC TGAACGAGAA ACGTAAATG ATATAAATAT CAATATATTA AATTAGATTT	
301	TGCATAAAAA ACAGACTACA TAATACTGTA AAACACAACA TATCCAGTCA CTATGGCGGC	
361	CGCTAAGTTG GCAGCATCAC CCGACGCACT TTGCGCCGAA TAAATACCTG TGACGGAAGA	
421	TCACTTCGCA GAATAAATAA ATCTGGTGT CCCTGTTGAT ACCGGGAAGC CCTGGGCCAA	
481	CTTTTGGCGA AAATGAGACG TTGATCGGCA CGTAAGAGGT TCCAACTTTC ACCATAATGA	
541	AATAAGATCA CTACCGGGCG TATTTTGTGA GTTATCGAGA TTTTCAGGAG CTAAGGAAGC	
601	TAAAAATGAG AAAAAATCA CTGGATATAC CACCGTTGAT ATATCCCAAT GGCATCGTAA	
661	AGAACATTTT GAGGCATTTT AGTCAGTTGC TCAATGTACC TATAACCAGA CCGTTCAGCT	
721	GGATATTACG GCCTTTTAA AGACCGTAAA GAAAAATAAG CACAAGTTTT ATCCGGCCTT	
781	TATTCACATT CTTGCCCCGC TGATGAATGC TCATCCGGA TTCCGTATGG CAATGAAAGA	
841	CGGTGAGCTG GTGATATGGG ATAGTGTTCA CCCTTGTTAC ACCGTTTTC ATGAGCAAAC	
901	TGAAACGTTT TCATCGCTCT GGAGTGAATA CCACGACGAT TTCCGGCAGT TTCTACACAT	
961	ATATTCGCAA GATGTGGCGT GTTACGGTGA AAACCTGGCC TATTTCCCTA AAGGGTTTAT	
1021	TGAGAATATG TTTTTCGTCT CAGCCAATCC CTGGGTGAGT TTCACCAGT TTGATTTAAA	
1081	CGTGGCCAAAT ATGGACAAC TCTTCGCCCC CGTTTTACC ATGGGCAAAT ATTATACGCA	
1141	AGGCGACAAG GTGCTGATGC CGCTGGCGAT TCAGGTTTCA CATGCCGTCT GTGATGGCTT	
1201	CCATGTCGGC AGAATGCTTA ATGAATTACA ACAGTACTGC GATGAGTGGC AGGGCGGGGC	
1261	GTAAGATCTT GGATCCGGCT TACTAAAAGC CAGATAACAG TATGCGTAT TGCGCGCTGA	
1321	TTTTTGGCGT ATAAGAATAT ATACTGATAT GTATACCCGA AGTATGTCAA AAAGAGGTGT	
1381	GCTATGAAGC AGCGTATTAC AGTGACAGTT GACAGCGACA GCTATCAGTT GCTCAAGGCA	
1441	TATATGATGT CAATATCTCC GGTCTGGTAA GCACAACCAT GCAGAATGAA GCGGTCGTC	
1501	TGCGTGCCGA ACGCTGGAAA GCGGAAAATC AGGAAGGGAT GGCTGAGGTC GCGCGTTTA	
1561	TTGAAATGAA CGGCTCTTTT GCTGACGAGA ACAGGGACTG GTGAAATGCA GTTTAAGGTT	
1621	TACACCTATA AAAGAGAGAG CCGTTATCGT CTGTTTGTGG ATGTACAGAG TGATATTATT	
1681	GACACGCCCC GCGACGGAT GGTGATCCCC CTGGCCAGTG CACGCTGCT GTCAGATAAA	
1741	GTCTCCCCGT AACTTTACCC GGTGGTGCAT ATCGGGGATG AAAGCTGGCG CATGATGACC	
1801	ACCGATATGG CCAGTGTGCC GGTCTCCGTT ATCGGGGAAG AAGTGGCTGA TCTCAGCCAC	
1861	CGCGAAAATG ACATCAAAAA CGCCATTAAC CTGATGTTCT GGGGAATATA AATGTCAGGC	
1921	TCCCTTATAC ACAGCCAGTC TGCAGGTCGA CCATAGTGAC TGGATATGTT GTGTTTTACA	
1981	GTATTATGTA GTCTGTTTTT TATGCAAAAG TGCTAATTTA ATATATTGAT ATTTATATCA	
2041	TTTACGTTT CTCGTTTCA GCCTTTGTAC AAAGTGGTGA TGGGAACCTC AGTTCAGTAG	
2101	TCGATCCCGC GGCCGCTTTC GAACCTAGGC AAGCATGCGG GCGGAGTGG TAATTAATTG	
2161	AATTACATCC CTACGCAAAC GTTTTACGGC CGCCGCTGGC GCGGCGGCC GCGGCGCCGT	
2221	CTTGGCCGT TGCAGGCCAC TCCGCTGGCT CCCGTCGTCC CCGACTTCCA GGCCCGCAG	
2281	ATGCAGCAAC TCATCAGCGC CGTAAATGCG CTGACAATGA GACAGAACGC AATTGCTCCT	
2341	GCTAGGAGCT TAATTCGACG AATAATTGGA TTTTATTATT ATTTTGCAAT TGGTTTTTAA	
2401	TATTTCCAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA-	

FIGURE 29B

65/240

2461 AAAAAAAAAA AAAAAAACTA GAAATCGCGA TTTCTAGTCT GCATTAATGA ATCGGCCAAC
2521 GCGCGGGGAG AGGCGGTTTG CGTATTGGGC GCTCTTCCGC TTCCTCGCTC ACTGACTCGC
2581 TGCGCTCGGT CGTTCCGGCTG CGGCGAGCGG TATCAGCTCA CTCAAAGGCC GTAATACGGT
2641 TATCCACAGA ATCAGGGGAT AACGCAGGAA AGAACATGTG AGCAAAAGGC CAGCAAAAGG
2701 CCAGGAACCG TAAAAAGGCC GCGTTGTGTG CGTTTTTCCA TAGGCTCCGC CCCCCTGACG
2761 AGCATCACAA AAATCGACGC TCAAGTCAGA GGTGGCGAAA CCCGACAGGA CTATAAAGAT
2821 ACCAGGCGTT TCCCCCTGGA AGCTCCCTCG TCGCTCTCC TGTTCCGACC CTGCCGCTTA
2881 CCGGATACCT GTCCGCCTTT CTCCCTTCGG GAAGCGTGGC GCTTTCTCAA TGCTCGCGCT
2941 GTAGGTATCT CAGTTCGGTG TAGGTCGTTT GCTCCAAGCT GGGCTGTGTG CACGAACCCC
3001 CCGTTCAGCC CGACCGCTGC GCCTTATCCG GTAACATATCG TCTTGAGTCC AACCCGGTAA
3061 GACACGACTT ATCGCCACTG GCAGCAGCCA CTGGTAACAG GATTAGCAGA GCGAGGTATG
3121 TAGGCGGTGC TACAGAGTTC TTGAAGTGGT GGCCTAACTA CGGCTACACT AGAAGGACAG
3181 TATTTGGTAT CTGCGCTCTG CTGAAGCCAG TTACCTTCGG AAAAAGAGTT GGTAGCTCTT
3241 GATCCGGCAA ACAAACCACC GCTGGTAGCG GTGGTTTTTT TGTTTGCAAG CAGCAGATTA
3301 CGCGCAGAAA AAAAGGATCT CAAGAAGATC CTTTGATCTT TTCTACGGGG TCTGACGCTC
3361 AGTGAACGA AAACTCACGT TAAGGGATTT TGGTCATGAG ATTATCAAAA AGGATCTTCA
3421 CCTAGATCCT TTAAATTAAT AAATGAAGTT TTAATCAAT CTAAAGTATA TATGAGTAAA
3481 CTTGGTCTGA CAGTTACCAA TGCTTAATCA GTGAGGCACC TATCTCAGCG ATCTGTCTAT
3541 TTCGTTTCATC CATAGTTGCC TGACTCCCCG TCGTGTAGAT AACTACGATA CGGGAGGGCT
3601 TACCATCTGG CCCCAGTGTG GCAATGATAC CGCGAGACCC ACGCTCACCG GCTCCAGATT
3661 TATCAGCAAT AAACCAGCCA CCGGGAAGGG CCGAGCGCAG AAGTGGTCCT GCAACTTTAT
3721 CCGCCTCCAT CCAGTCTATT AATTGTTGCC GGAAGCTAG AGTAAGTAGT TCGCCAGTTA
3781 ATAGTTTGCG CAACGTTGTT GCCATTGCTA CAGGCATCGT GGTGTCACGC TCGTCGTTTG
3841 GTATGGCTTC ATTCAGCTCC GGTTCCTAAC GATCAAGGCG AGTTACATGA TCCCCATGT
3901 TGTGCAAAAA AGCGGTTAGC TCCTTCGGTC CTCCGATCGT TGTCAGAAGT AAGTTGGCCG
3961 CAGTGTTATC ACTCATGGTT ATGGCAGCAC TGCAATAATC TCTTACTGTC ATGCCATCCG
4021 TAAGATGCTT TTCTGTGACT GGTGAGTACT CAACCAAGTC ATTCTGAGAA TAGTGTATGC
4081 GCGGACCGAG TTGCTCTTGC CCGGCGTCAA TACGGGATAA TACCGGCCA CATAGCAGAA
4141 CTTTAAAGT GCTCATCAIT GGAAAACGTT CTTGGGGCG AAAACTCTCA AGGATCTTAC
4201 CGCTGTTGAG ATCCAGTTCG ATGTAACCCA CTCGTGCACC CAACTGATCT TCAGCATCTT
4261 TTACTTTTAC CAGCGTTTCT GGGTGAGCAA AAACAGGAAG GCAAAATGCC GCAAAAAAGG
4321 GAATAAGGGC GACACGGAAT TGTTGAATAC TCATACTCTT CTTTTTCAA TATTATTGAA
4381 GCATTTATCA GGGTTATTGT CTCATGAGCG GATACATATT TGAATGTATT TAGAAAAATA
4441 AACAAATAGG GGTTCGCGC ACATTTCCCC GAAAAGTGCC ACCTGACGTC TAAGAAACCA
4501 TTATTATCAT GACATTAACC TATAAAAAA GGCGTATCAC GAGGCCCTTT CGTCTCGCGC
4561 GTTTCGGTGA TGACGGTGAA AACCTCTGAC ACATGCAGCT CCGGAGACG GTCACAGCTT
4621 CTGTCTAAGC GGATGCCGGG AGCAGACAAG CCCGTCAGGG CGCGTCAGCG GGTGTTGGCG
4681 GGTGTCGGGG CTGGCTTAAC TATGCGGCAT CAGAGCAGAT TGTAAGTAGA GTGCACCATA
4741 TCGACGCTCT CCCTTATGCG ACTCCTGCAT TAGGAAGCAG CCCAGTACTA GGTGAGGCC
4801 GTTGAGCACC GCCGCCGCAA GGAATGTGTC ATGCAAGGAG ATGGCGCCCA ACAGTCCCCC
4861 GGCCACGGGG CCTGCCACCA TACCCACGCC GAAACAAGCG CTCATGAGCC CGAAGTGGCG
4921 AGCCCCGATCT TCCCCATCGG TGATGTCCGG GATATAGGCG CCAGCAACCG CACCTGTGGC
4981 GCCGGTGATG CCGGCCACGA TCGTCCGGC GTAGAGGATC TGGCTAGCGA TGACCCTGCT
5041 GATTGGTTTC CTGACCATTT CCGGGGTGCG GAACGGCGTT ACCAGAACT CAGAAGGTTT
5101 GTCCAACCAA ACCGACTCTG ACGGCAGTTT ACGAGAGAGA TGATAGGGTC TGCTTCAGTA
5161 AGCCAGATGC TACACAATTA GGCTTGATCA TATTGTCGTT AGAACGCGGC TACAATTAAT
5221 ACATAACCTT ATGTATCATA CACATACGAT TTAGGTGACA CTATAGATGG CGGATGTGTG
5281 ACATACACGA CGCCAAAAGA TTTTGTCCA GCTCCTGCCA CCTCCGCTAC GCGAGAGATT
5341 AACCACCCAC GATGGCCGCC AAAGTGCATG TTGATATTGA GGCTGACAGC CCATTTCATCA
5401 AGTCTTTGCA GAAGGCATTT CCGTCGTTCC AGGTGGAGTC ATTGCAGGTC ACACCAAATG
5461 ACCATGCAAA TGCCAGAGCA TTTTCGCACC TGGCTACCAA ATTGATCGAG CAGGAGACTG
5521 ACAAAGACAC ACTCATCTTG GATATCGGCA GTGCGCCTTC CAGGAGAATG ATGTCTACGC
5581 ACAAATACCA CTGCGTATGC CCTATGCGCA GCGCAGAAGA CCCCAGAAAG CTCGATAGCT
5641 ACGCAAAGAA ACTGGCAGCG GCCTCCGGGA AGGTGCTGGA TAGAGAGATC GCAGGAAAAA
5701 TCACCGACCT GCAGACCGTC ATGGCTACGC CAGACGCTGA ATCTCCTACC TTTTGCCTGC
5761 ATACAGACGT CACGTGTCTG ACGGCAGCCG AAGTGGCCGT ATACCAGGAC GTGTATGCTG
5821 TACATGCACC AACATCGCTG TACCATCAGG CGATGAAAGG TGTCAGAACG GCGTATTGGA
5881 TTGGGTTTGA CACCACCCCG TTTATGTTTG ACGCGCTAGC AGGCGCGTAT CCAACCTACG-

FIGURE 29C

66/240

5941 CCACAACTG GCGGACGAG CAGGTGTTAC AGGCCAGGAA CATAGGACTG TGTGCA3CAT
6001 CCTTGACTGA GGGAAAGACTC GGCAAAGTGT CCATTCTCCG CAAGAAGCAA TTGAAACCTT
6061 GCGACACAGT CATGTTCTCG GTAGGATCTA CATTGTACAC TGAGAGCAGA AAGCTACTGA
6121 GGAGCTGGCA CTTACCCTCC GTATTCCACC TGAAAGGTAA ACAATCCTTT ACCTGTAGGT
6181 GCGATACCAT CGTATCATGT GAAGGGTACG TAGTTAAGAA AATCACTATG TGCCCCGGCC
6241 TGTACCGTAA AACGGTAGGG TAGGCCGTGA CGTATCACGC GGAGGGATTG CTAGTGTGCA
6301 AGACCACAGA CACTGTCAAA GGAGAAAGAG TCTCATTCCC TGTATGCACC TACGTC3CCT
6361 CAACCATCTG TGATCAAATG ACTGGCATACT TAGCGACCGA CGTCACACCG GAGGAC3CAC
6421 AGAAGTTGTT AGTGGGATTG AATCAGAGGA TAGTTGTGAA CGGAAGAACA CAGCGAAACA
6481 CTAACACGAT GAAGAACTAT CTGCTTCCGA TTGTGGCCGT CGCATTAGC AAGTGG3CGA
6541 GGGAAATACAA GGCAGACCTT GATGATGAAA AACCTCTGGG TGTCCGAGAG AGGTCACTTA
6601 CTTGCTGCTG CTTGTGGGCA TTTAAAACGA GGAAGATGCA CACCATGTAC AAGAAACCGAG
6661 ACACCCAGAC AATAGTGAAG GTGCCCTCAG AGTTTAACTC GTTCGTATC CCGAGCCTAT
6721 GGTCTACAGG CCTCGCAATC CCAGTCAGAT CACGCATTAA GATGCTTTTG GAACAAGA
6781 CCAAGCGAGA GTTAATACCT GTTCTCGACG CGTCGTCAGC CAGGGATGCT GAACAAGAGG
6841 AGAAGGAGAG GTTGGAGGCC GAGCTGACTA GAGAAGCCTT ACCACCCCTC GTCCCCATCG
6901 CGCCGGCGGA GACGGGAGTC GTCGACGTCG ACGTTGAAGA ACTAGAGTAT CACGCA3GTG
6961 CAGGGGTGCT GGAAACACCT CGCAGCGCGT TGAAAGTCAC CGCACAGCCG AACGAC3TAC
7021 TACTAGGAAA TTACGTAGTT CTGTCCCCGC AGACCGTGCT CAAGAGCTCC AAGTTG3CCC
7081 CCGTGACCCC TCTAGCAGAG CAGGTGAAAA TAATAACACA TAACGGGAGG GCCGGCG3GT
7141 ACCAGGTGCA CGGATATGAC GGCAGGGTCC TACTACCATG TGGATCGGCC ATTCGG3TCC
7201 CTGAGTTTCA GGCTTTGAGC GAGAGCGCCA CTATGGTGTA CAACGAAAGG GAGTTC3TCA
7261 ACAGGAAACT ATACCATATT GCCGTTACG GACCCTCGCT GAACACCGAC GAGGAGAACT
7321 ACGAGAAAGT CAGAGCTGAA AGAACTGACG CCGAGTACGT GTTCGACGTA GATAAAAAAT
7381 GCTGCGTCAA GAGAGAGGAA GCGTCGGGTT TGGTGTGGT GGGAGAGCTA ACCAACC3CC
7441 CGTTCCATGA ATTCGCCTAC GAAGGGCTGA AGATCAGGCC GTCGGCACCA TATAAGACTA
7501 CAGTAGTAGG AGTCTTTGGG GTTCCGGGAT CAGGCAAGTC TGCTATTATT AAGAGCCTCG
7561 TGACCAAAAC CGATCTGGTC ACCAGCGGCA AGAAGGAGAA CTGCCAGGAA ATAGTTA3ACG
7621 ACGTGAAGAA GCACCGCGGG AAGGGGAAA GTAGGGAAA CAGTGA3TCC ATCTGCTAA
7681 ACGGGTGTG TCGTGCCGTG GACATCCTAT ATGTGGACGA GGCTTT3CGT TGCCAT3CCG
7741 GTACTCTGCT GGCCCTAATT GCTCTTGTTA AACCTCGGAG CAAAGTGGTG TTATGC3GAG
7801 ACCCAAGCA ATGCGGATTC TTCAATATGA TGCAGCTTAA GGTGA3CTC AACCAACA
7861 TCTGCACTGA AGTATGTCAT AAAAGTATAT CCAGACGTTG CACGCGTCCA GTCACG3CCA
7921 TCGTGTCTAC GTTGCACTAC GGAGGCAAGA TGCACACGAC CAACCCGTGC AACAA3CCA
7981 TAATCATAGA CACCACAGGA CAGACCAAGC CCAAGCCAGG AGACATCGTG TTAACATGCT
8041 TCCGAGGCTG GGCAAGCAG CTGCAGTTGG ACTACCGTGG ACACGAAGTC ATGAC3CAG
8101 CAGCATCTCA GGGCCTCACC CGCAAAGGGG TATACGCCGT AAGGCAGAAG GTGAAT3AAA
8161 ATCCCTTGTA TGCCCTGCG TCGGAGCAG TGAATGTACT GCTGACGCGC ACTGAG3ATA
8221 GGCTGGGTG GAAAACGCTG GCCGGCGATC CCTGGATTAA GGTCT3ATC AACATT3CAC
8281 AGGGTAACCT TACGGCCACA TTGGAAGAAT GGCAAGAAGA ACACGACAAA ATAATG3AGG
8341 TGATTGAAGG ACCGGCTGCG CCTGTGGACG CGTTCAGAA CAAAGCGAAC GTGTGT3GGG
8401 CGAAAAGCCT GGTGCCTGTC CTGGACACTG CCGGAATCAG ATTGACAGCA GAGGAGT3GA
8461 GCACCATAAT TACAGCATTT AAGGAGGACA GAGCTTACTC TCCAGTGGTG GCCTTGA3ATG
8521 AAATTTGCAC CAAGTACTAT GGAGTTGACC TGGACAGTGG CCTGTTT3CT GCCCGA3AGG
8581 TGTCCCTGTA TTACGAGAAC AACCCTGGG ATAACAGACC TGGTGAAGG ATGTAT3GAT
8641 TCAATGCCGC AACAGCTGCC AGGCTGGAAG CTAGACATAC CTTCTGAAG GGGCAGT3GC
8701 ATACGGGCAA GCAGGCAGTT ATCGCAGAAA GAAAAATCCA ACCGCTTTCT GTGCTG3ACA
8761 ATGTAATTC TATCAACCGC AGGCTGCCGC ACGCCCTGGT GGCTGAGTAC AAGACG3TTA
8821 AAGGCAGTAG GGTGAGTGG CTGGTCAATA AAGTAAGAGG GTACCACGTC CTGCTG3TGA
8881 GTGAGTACAA CCTGGCTTTG CCTCGACGCA GGGTCACTTG GTTGTACCCG CTGAAT3TCA
8941 CAGGCGCCGA TAGGTGCTAC GACCTAAGTT TAGGACTGCC GGCTGACGCC GGCAGG3TCC
9001 ACTTGGTCTT TGTGAACATT CACACGGAAT TCAGAATCCA CCACTACCAG CAGTGT3TCG
9061 ACCACGCCAT GAAGCTGCAG ATGCTTGGGG GAGATGCGCT ACGACTGCTA AAACCC3GCG
9121 GCATCTTGAT GAGAGCTTAC GGATACGCCG ATAAAATCAG CGAAGCCGTT GTTTCT3CCT
9181 TAAGCAGAAA GTTCTCGTCT GCAAGAGTGT TCGCCCCGGA TTGTGTCACC AGCAAT3ACAG
9241 AAGTGTCTT GCTGTTCTCC AACTTTGACA ACGGAAAGAG ACCCTCTACG CTACAC3AGA
9301 TGAATACCAA GCTGAGTGCC GTGTATGCCG GAGAAGCCAT GCACACGGCC GGGTGT3CAC
9361 CATCCTACAG AGTTAAGAGA GCAGACATAG CCACGTGCAC AGAAGCGGCT GTGGTTA3ACG-

FIGURE 29A

67/240

9421 CAGCTAACGC CCGTGGAACT GTAGGGGATG GCGTATGCAG GGCCGTGGCG AAGAAATGGC
9481 CGTCAGCCTT TAAGGGAGCA GCAACACCAG TGGGCACAAT TAAAACAGTC ATGTGCGGCT
9541 CGTACCCCGT CATCCACGCT GTAGCGCCTA ATTTCTCTGC CACGACTGAA GCGGAAGGGG
9601 ACCGCGAATT GGCCGCTGTC TACCGGGCAG TGGCCGCCGA AGTAAACAGA CTGTCACTGA
9661 GCAGCGTAGC CATCCCGCTG CTGTCCACAG GAGTGTTTCA GCGCGGAAGA GATAGGCTGC
9721 AGCAATCCCT CAACCATCTA TTCACAGCAA TGGACGCCAC GGACGCTGAC GTGACCATCT
9781 ACTGCAGAGA CAAAAGTTGG GAGAAGAAAA TCCAGGAAGC CATTGACATG AGGACGCTG
9841 TGGAGTTGCT CAATGATGAC GTGGAGCTGA CCACAGACTT GGTGAGAGTG CACCCGGACA
9901 GCAGCCTGGT GGGTCGTAAG GGCTACAGTA CCACTGACGG GTCGCTGTAC TCGTACTTTG
9961 AAGGTACGAA ATTCAACCAG GCTGCTATTG ATATGGCAGA GATACTGACG TTGTGGCCCA
10021 GACTGCAAGA GGCAAACGAA CAGATATGCC TATACGCGCT GGGCGAAACA ATGGACAACA
10081 TCAGATCCAA ATGTCCGGTG AACGATTCCG ATTCATCAAC ACCTCCCAGG ACAGTGCCCT
10141 GCCTGTGCGG CTACGCAATG ACAGCAGAAC GGATCGCCCG CCTTAGGTCA CACCAAGTTA
10201 AAAGCATGGT GGTTTGCTCA TCTTTTCCCC TCCCGAAATA CCATGTAGAT GCGGTGACGA
10261 AGGTAAAGTG CGAGAAGGTT CTCTGTTCG ACCCGACGGT ACCTTCAGTG GTTAGTCCGC
10321 GGAAGTATGC CGCATCTACG ACGGACCACT CAGATCGGTC GTTACGAGGG TTTGACTTGG
10381 ACTGGACCAC CGACTCGTCT TCCACTGCCA GCGATACCAT GTCGCTACCC AGTTTGCAGT
10441 CGTGTGACAT CGACTCGATC TACGAGCCAA TGGCTCCCAT AGTAGTGACG GCTGACGTAC
10501 ACCCTGAACC CGCAGGCATC GCGGACCTGG CGGCAGATGT GCACCCTGAA CCCGCAGACC
10561 ATGTGGACCT GGAGAACCCG ATTCTCTCCAC CGCGCCCGAA GAGAGCTGCA TACCTTGCTT
10621 CCCGCGCGCG GGAGCGACCG GTGCCGCGCG CGAGAAAGCC GACGCTGCC CCAAGGACTG
10681 CGTTTAGGAA CAAGCTGCCT TTGACGTTTC GCGACTTTGA CGAGCACGAG GTCGATGCGT
10741 TGGCTCCGG GATTACTTTC GGAGACTTCG ACGACGTCCT GCGACTAGGC CGCGCGGGTG
10801 CATATATTTT CTCTCGGAC ACTGGCAGCG GACATTTACA AAAAAATCC GTTAGGCAGC
10861 ACAATCTCCA GTGCGCACA CTGGATGCGG TCCAGGAGGA GAAAATGTAC CCGCCAAAT
10921 TGGATACTGA GAGGGAGAAG CTGTTGCTGC TGAATATGCA GATGCACCA TCGGAGGCTA
10981 ATAAGAGTCG ATACCACTCT CGCAAAGTGG AGAATATGAA AGCCACGGTG GTGGACAGGC
11041 TCACATCGGG GGCCAGATTG TACACGGGAG CGGACGTAGG CCGCATACCA ACATACGCGG
11101 TTCGGTACCC CCGCCCGTG TACTCCCCTA CCGTGATCGA AAGATTCTCA AGCCCCGATG
11161 TAGCAATCGC AGCGTGCAAC GAATACCTAT CCAGAAATTA CCAACAGTG GCGTCGTACC
11221 AGATAACAGA TGAATACGAC GCATACTTGG ACATGGTTGA CGGGTCGGAT AGTTGCTTGG
11281 ACAGAGCGAC ATTCTGCCCG GCGAAGCTCC GGTGCTACCC GAAACATCAT GCGTACCACC
11341 AGCCGACTGT ACGCAGTGCC GTCCCGTCAC CCTTTCAGAA CACACTACAG AACGTGCTAG
11401 CGGCTGCCAC CAAGAGAAAC TGCAACGTCA CGCAAATGCG AGAACTACCC ACCATGGACT
11461 CGGCAGTGTT CAACGTGGAG TGCTTCAAGC GCTATGCCTG CTCCGGAGAA TATTGGGAAG
11521 AATATGCTAA ACAACCTATC CGGATAACCA CTGAGAACAT CACTACCTAT GTGACCAAAT
11581 TGAAAGGCCC GAAAGCTGCT GCCTTGTTTCG CTAAGACCCA CAACTTGTTT CCGCTGCAGG
11641 AGGTTCCTCAT GGACAGATTG ACGGTCGACA TGAACGAGA TGTCAAAGTC ACTCCAGGGA
11701 CGAAACACAC AGAGGAAAGA CCCAAAGTCC AGGTAATTCA AGCAGCGGAG CCATTGGCGA
11761 CCGCTTACCT GTGCGGCATC CACAGGGAAT TAGTAAGGAG ACTAAATGCT GTGTTACGCC
11821 CTAACGTGCA CACATTGTTT GATATGTGCG CCGAAGACTT TGACGCGATC ATCGCCTCTC
11881 ACTTCCACCC AGGAGACCCG GTTCTAGAGA CGGACATTGC ATCATTCGAC AAAAGCCAGG
11941 ACGACTCCTT GGCTCTTACA GGTTTAATGA TCCTCGAAGA TCTAGGGGTG GATCAGTACC
12001 TGCTGGACTT GATCGAGGCA GCCTTTGGGG AAATATCCAG CTGTACCTA CCAACTGGCA
12061 CGCGCTTCAA GTTCGGAGCT ATGATGAAAT CGGGCATGTT TCTGACTTTG TTTATTAACA
12121 CTGTTTTGAA CATCACCATA GCAAGCAGGG TACTGGAGCA GAGACTCACT GACTCCGCCT
12181 GTGCGGCCTT CATCGGCGAC GACAACATCG TTCACGGAGT GATCTCCGAC AAGCTGATGG
12241 CGGAGAGGTG CGCGTCGTGG GTCAACATGG AGGTGAAGAT CATTGACGCT GTCATGGGCG
12301 AAAAACCCCC ATATTTTGT GGGGGATTCA TAGTTTTTGA CAGCGTCACA CAGACCCCTT
12361 GCGGTGTTTC AGACCCACTT AAGCGCCTGT TCAAGTTGGG TAAGCCGCTA ACAGCTGAAG
12421 ACAAGCAGGA CGAAGACAGG CGACGAGCAC TGAGTGACGA GGT

FIGURE 29E

69/240

pDEST10 6708 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
23..152		Ppolh
461..337		attR1
711..1370		CmR
1490..1574		inactivated ccdA
1712..2017		ccdB
2058..2182		attR2
3394..4369		ampR
4510..5164		ori
5658..62		genR

1	CCCCGGATGA	AGTGGTTCGC	ATCCTCGGTT	TTCTGGAAGG	CGAGCATCGT	TTGTTTCGCCC
61	AGGACTCTAG	CTATAGTTCT	AGTGGTTGGC	TACGTATACT	CCGGAATATT	AATAGATCAT
121	GGAGATAATT	AAAATGATAA	CCATCTCGCA	AATAAATAAG	TATTTTACTG	TTTTTCGTAAC
181	AGTTTTGTAA	TAAAAAAACC	TATAAATATT	CCGGATTATT	CATACCGTCC	CACCATCGGG
241	CGCGGATCTC	GGTCCGAAAC	CATGTCGTAC	TACCATCACC	ATCACCATCA	CGATTACGAT
301	ATCCCAACGA	CCGAAAACCT	GTATTTTCAG	GGCATCACAA	GTTTGTACAA	AAAAGCTGAA
361	CGAGAAACGT	AAAATGATAT	AAATATCAAT	ATATTAAATT	AGATTTTGCA	TAAAAAACAG
421	ACTACATAAT	ACTGTAAAAC	ACAACATATC	CAGTCACTAT	GGCGGCCGCT	AAGTTGGCAG
481	CATCACCCGA	CGCACTTTGC	GCCGAATAAA	TACCTGTGAC	GGAAGATCAC	TTCCGAGAAT
541	AAATAAATCC	TGGTGTCCCT	GTTGATACCG	GGAAGCCCTG	GGCCAACCTT	TGGCGAAAAAT
601	GAGACGTTGA	TCGGCACGTA	AGAGGTTCCA	ACTTTCACCA	TAATGAAATA	AGATCACTAC
661	CGGGCGTATT	TTTTGAGTTA	TCGAGATTTT	CAGGAGCTAA	GGAAGCTAAA	ATGGAGAAAA
721	AAATCACTGG	ATATACCACC	GTTGATATAT	CCCAATGGCA	TCGTAAAGAA	CATTTTGAGG
781	CATTTTCAGT	AGTTGCTCAA	TGTACCTATA	ACCAGACCGT	TCAGCTGGAT	ATTACGGCCT
841	TTTTAAAGAC	CGTAAAGAAA	AATAAGCACA	AGTTTTATCC	GGCCTTTATT	CACATTCCTG
901	CCCGCCTGAT	GAATGCTCAT	CCGGAATTCC	GTATGGCAAT	GAAAGACGGT	GAGCTGGTGA
961	TATGGGATAG	TGTTACCCCT	TGTTACACCG	TTTTCATGTA	GCAAACTGAA	ACGTTTTTCAT
1021	CGCTCTGGAG	TGAATACCAC	GACGATTTCC	GGCAGTTTCT	ACACATATAT	TCGCAAGATG
1081	TGGCGTGTTA	CGGTGAAAAC	CTGGCCTATT	TCCCTAAAGG	GTTTATTGAG	AATATGTTTT
1141	TCGTCTCAGC	CAATCCCTGG	GTGAGTTTCA	CCAGTTTTGA	TTTAAACGTG	GCCAATATGG
1201	ACAACCTTCT	CGCCCCGTTT	TTCAACATGG	GCAAATATTA	TACGCAAGGC	GACAAGGTGC
1261	TGATGCCGCT	GGCGATTTCAG	GTTTCATCAT	CCGTCTGTGA	TGGCTTCCAT	GTCGGCAGAA
1321	TGCTTAATGA	ATTACAACAG	TACTGCGATG	AGTGGCAGGG	CGGGGCGTAA	ACGCGTGGAT
1381	CCGGCTTACT	AAAAGCCAGA	TAACAGTATG	CGTATTTGCG	CGCTGATTTT	TGCGGTATAA
1441	GAATATATAC	TGATATGTAT	ACCCGAAGTA	TGTCAAAAAG	AGGTGTGCTA	TGAAGCAGCG
1501	TATTACATG	ACAGTTGACA	GCGACAGCTA	TCAGTTGCTC	AAGGCATATA	TGATGTCAAT
1561	ATCTCCGGTC	TGGTAAGCAC	AACCATGCAG	AATGAAGCCC	GTCGTCTGCG	TGCCGAACGC
1621	TGGAAAGCGG	AAAATCAGGA	AGGGATGGCT	GAGGTCGCCC	GGTTTATTGA	AATGAACGGC
1681	TCTTTTGCTG	ACGAGAACAG	GGACTGGTGA	AATGCAGTTT	AAGGTTTACA	CCTATAAAAG
1741	AGAGAGCCGT	TATCGTCTGT	TTGTGGATGT	ACAGAGTGAT	ATTATTGACA	CGCCCCGGCG
1801	ACGGATGGTG	ATCCCCCTGG	CCAGTGCACG	TCTGCTGTCA	GATAAAGTCT	CCCGTGAAC
1861	TTACCCGGTG	GTGCATATCG	GGGATGAAAG	CTGGCGCATG	ATGACCACCG	ATATGGCCAG
1921	TGTGCCGGTC	TCCGTTATCG	GGGAAGAAGT	GGCTGATCTC	AGCCACCGCG	AAAATGACAT
1981	CAAAAACGCC	ATTAACCTGA	TGTTCTGGGG	AATATAAATG	TCAGGCTCCC	TTATACACAG
2041	CCAGTCTGCA	GGTCGACCAT	AGTGACTGGA	TATGTTGTGT	TTTACAGTAT	TATGTAGTCT
2101	GTTTTTTATG	CAAAATCTAA	TTTAATATAT	TGATATTTAT	ATCATTTTAC	GTTTCTCGTT
2161	CAGCTTTTCT	GTACAAAGTG	GTGATGCCAT	GGATCCGGAA	TTCAAAGGCC	TACGTCGACG
2221	AGCTCAACTA	GTGCGGCCGC	TTTCGAATCT	AGAGCCTGCA	GTCTCGAGGC	ATGCGGTACC
2281	AAGCTTGTCT	AGAAGTACTA	GAGGATCATA	ATCAGCCATA	CCACATTTGT	AGAGGTTTTA
2341	CTTGCTTTTAA	AAAACCTCCC	ACACCTCCCC	CTGAACCTGA	AACATAAAAT	GAATGCAATT
2401	GTTGTTGTTA	ACTTGTTTAT	TGCAGCTTAT	AATGGTTACA	AATAAAGCAA	TAGCATCACA
2461	AATTTTCACAA	ATAAAGCATT	TTTTTCACATG	CATTCTAGTT	GTGGTTTGTG	CAAACTCATC
2521	AATGTATCTT	ATCATGTCTG	GATCTGATCA	CTGCTTGAGC	CTAGGAGATC	CGAACCAGAT
2581	AAGTGAAATC	TAGTTCCAAA	CTATTTTGTC	ATTTTTAATT	TTCGTATTAG	CTTACGACGC-

FIGURE 30B

70/240

2641 TACACCCAGT TCCCATCTAT TTTGTCACTC TTCCCTAAAT AATCCTTAAA AACTCCATTT
 2701 CCACCCCTCC CAGTTCCCAA CTATTTTGTC CGCCACAGC GGGGCATTTT TCTTCCTGTT
 2761 ATGTTTTTAA TCAAACATCC TGCCAACTCC ATGTGACAAA CCGTCATCTT CGGCTACTTT
 2821 TTCTCTGTCA CAGAATGAAA ATTTTCTGT CATCTCTTCG TTATTAATGT TTGTAATTGA
 2881 CTGAATATCA ACGCTTATTT GCAGCCTGAA TGGCGAATGG GACGCGCCCT GTAGCGGCGC
 2941 ATTAAGCGCG GCGGGTGTGG TGGTTACGG CAGCGTGACC GCTACACTTG CCAGCGCCCT
 3001 AGCGCCCCGCT CCTTTTCGCTT TCTTCCCTTC CTTTCTCGCC AC GTTTCGCCG GCTTTCCCGG
 3061 TCAAGCTCTA AATCGGGGGC TCCCTTTAGG GTTCCGATTT AGTGCTTTAC GGCACCTCGA
 3121 CCCCCAAAAA CTTGATTAGG GTGATGGTTC ACGTAGTGGG CCATCGCCCT GATAGACGGT
 3181 TTTTCGCCCT TTGACGTTGG AGTCCACGTT CTTTAATAGT GGACTCTTGT TCCAACTGG
 3241 AACAACACTC AACCCTATCT CGGTCTATTC TTTTGATTTA TAAGGGATTT TGCCGATTTT
 3301 GGCCTATTGG TTAAAAATG AGCTGATTTA ACAAAAATTT AACGCGAATT TTAACAAAAT
 3361 ATTAACGTTT ACAATTTTCT GTGGCACTTT TCGGGGAAAT GTGCGCGGAA CCCCTATTTG
 3421 TTTATTTTTC TAAATACATT CAAATATGTA TCCGCTCATG AGACAATAAC CCTGATAAAT
 3481 GCTTCAATAA TATTGAAAAA GGAAGAGTAT GAGTATTTCA CATTTCCGTG TCGCCCTTAT
 3541 TCCCTTTTTT GCGGCATTTT GCCTTCCTGT TTTTGCTCAC CCAGAAACGC TGGTGAAAGT
 3601 AAAAGATGCT GAAGATCAGT TGGGTGCACG ACTGGGTAC ATCGAACTGG ATCTCAACAG
 3661 CGGTAAGATC CTTGAGAGTT TTCGCCCCGA AGAACGTTTT CCAATGATGA GCACCTTTAA
 3721 AGTTCTGCTA TGTGGCGCGG TATTATCCCG TATTGACGCC GGGCAAGAGC AACTCGGTGCG
 3781 CCGCATACAC TATTCTCAGA ATGACTTGGT TGAGTACTCA CCAGTCACAG AAAAGCATCT
 3841 TACGGATGGC ATGACAGTAA GAGAATTATG CAGTGCTGCC ATAACCATGA GTGATAACAC
 3901 TGCGGCCAAC TTACTTCTGA CAACGATCGG AGGACCGAAG GAGCTAACCG CTTTTTTGCA
 3961 CAACATGGGG GATCATGTAA CTCGCCCTGA TCGTTGGGAA CCGGAGCTGA ATGAAGCCAT
 4021 ACCAAACGAC GAGCGTGACA CCACGATGCC TGTAGCAATG GCAACAACGT TCGCCAAACT
 4081 ATTAAGTGGC GAACTACTTA CTCTAGCTTC CCGGCAACAA TTAATAGACT GGATGGAGGC
 4141 GGATAAAGTT GCAGGACCAC TTCTGCGCTC GGCCCTTCCG GCTGGCTGGT TTATTGCTGA
 4201 TAAATCTGGA GCCGGTGAGC GTGGGTCTCG CGGTATCATT GCAGCACTGG GGCCAGATGG
 4261 TAAGCCCTCC CGTATCGTAG TTATCTACAC GACGGGGAGT CAGGCAACTA TGGATGAACG
 4321 AAATAGACAG ATCGCTGAGA TAGGTGCCTC ACTGATTAAG CATTGGAAC TGTCAGACCA
 4381 AGTTTACTCA TATATACTTT AGATTGATTT AAAAATTCAT TTTTAATTTA AAAGGATCTA
 4441 GGTGAAGATC CTTTTTGATA ATCTCATGAC CAAAATCCCT TAACGTGAGT TTTCTGCG
 4501 CTGAGCGTCA GACCCCGTAG AAAAGATCAA AGGATCTTCT TGAGATCCTT TTTTCTGCG
 4561 CGTAATCTGC TGCTTGCAAA CAAAAAACC ACCGCTACCA GCGGTGGTTT GTTTGCCGGA
 4621 TCAAGAGCTA CCAACTCTTT TTCCGAAGGT AACTGGCTTC AGCAGAGCGC AGATAACAAA
 4681 TACTGTCTT CTAGTGTAGC CGTAGTTAGG CCACCACTTC AAGAACTCTG TAGCACCGCC
 4741 TACATACCTC GCTCTGCTAA TCCTGTTACC AGTGGCTGCT GCCAGTGGCG ATAAGTCGTG
 4801 TCTTACCGGG TTGGACTCAA GACGATAGTT ACCGGATAAG GCGCAGCGGT CGGGCTGAAC
 4861 GGGGGTTCG TGACACAGC CCAGCTTGGG GCGAACGACC TACACCGAAC TGAGATACCT
 4921 ACAGCGTGAG CATTGAGAAA GCGCCACGCT TCCCGAAGGG AGAAAGGCGG ACAGGTATCC
 4981 GGTAAGCGGC AGGGTCGGAA CAGGAGAGCG CACGAGGGAG CTTCCAGGGG GAAACGCTG
 5041 GTATCTTTAT AGTCCTGTG GGTTCGCCA CCTCTGACTT GAGCGTCGAT TTTTGTGATG
 5101 CTCGTAGGG GGGCGGAGCC TATGGAAAA CGCCAGCAAC GCGGCCTTTT TACGGTTCCT
 5161 GGCCTTTTGC TGGCCTTTTG CTCACATGTT CTTTCCTGCG TTATCCCTG ATTCTGTGGA
 5221 TAACCGTATT ACCGCTTTTG AGTGAGCTGA TACCGCTCGC CGCAGCCGAA CGACCGAGCG
 5281 CAGCGAGTCA GTGAGCGAGG AAGCGGAAGA GCGCCTGATG CGGTATTTTC TCCTTACGCA
 5341 TCTGTGCGGT ATTTACACC GCAGACCAGC CGCGTAACCT GGCAAAATCG GTTACGGTTG
 5401 AGTAATAAAT GGATGCCCTG CGTAAGCGGG TGTGGGCGGA CAATAAAGTC TTAAGTGA
 5461 CAAAATAGAT CTAAACTATG ACAATAAAGT CTTAAACTAG ACAGAATAGT TGTAAACTGA
 5521 AATCAGTCCA GTTATGCTGT GAAAAAGCAT ACTGGACTTT TGTATGGCT AAAGCAAACT
 5581 CTTCATTTTC TGAAGTGCAA ATTGCCCGTC GTATTAAAGA GGGGCGTGGC CAAGGGCATG
 5641 GTAAAGACTA TATTCGCGGC GTTGTGACAA TTTACCGAAC AACTCCGCGG CCGGGAAGCC
 5701 GATCTCGGCT TGAACGAATT GTTAGGTGGC GGTACTTGGG TCGATATCAA AGTGCATCAC
 5761 TTCTTCCCGT ATGCCCAACT TTGTATAGAG AGCCACTGCG GGATCGTCAC CGTAATCTGC
 5821 TTGCACGTAG ATCATAAG CACCAAGCGC GTTGGCTCA TGCTTGAGGA GATTGATGAG
 5881 CGCGGTGGCA ATGCCCTGCC TCCGGTGCTC GCCGAGACT GCGAGATCAT AGATATAGAT
 5941 CTCACTACGC GGCTGCTCAA ACCTGGGCAG AACGTAAGCC GCGAGAGCGC CAACAACCGC
 6001 TTCTTGGTGC AAGGCAGCAA GCGCGATGAA TGTCTTACTA CCGAGCAAGT TCCCGAGGTA
 6061 ATCGGAGTCC GGCTGATGTT GGGAGTAGGT GGCTACGCT CCGAACTCAC GACCGAAAAG-

FIGURE 30C

6121 ATCAAGAGCA GCCCGCATGG ATTTGACTTG GTCAGGGCCG AGCCTACATG TGCGAATGAT
6181 GCCCATACTT GAGCCACCTA ACTTTGTTTT AGGGCGACTG CCCTGCTGCG TAACATCGTT
6241 GCTGCTGCGT AACATCGTTG CTGCTCCATA ACATCAAACA TCGACCCACG GCGTAACGCG
6301 CTTGCTGCTT GGATGCCCGA GGCATAGACT GTACAAAAA ACAGTCATAA CAAGCCATGA
6361 AAACCGCCAC TGCGCCGTTA CCACCGCTGC GTTCGGTCAA GGTTCTGGAC CAGTTGCGTG
6421 AGCGCATACG CTACTTGCA TACAGTTTAC GAACCGAACA GGCTTATGTC AACTGGGTTT
6481 GTGCCTTCAT CCGTTTCCAC GGTGTGCGTC ACCCGGCAAC CTTGGGCAGC AGCGAAGTCG
6541 AGGCATTTCT GTCCTGGCTG GCGAACGAGC GCAAGGTTTC GGTCTCCACG CATCGTCAGG
6601 CATTGGCGGC CTTGCTGTTC TTCTACGGCA AGGTGCTGTG CACGGATCTG CCCTGGCTTC
6661 AGGAGATCGG AAGACCTCGG CCGTCGCGGC GCTTGCCGGT GGTGCTGA

72/240

Figure 31A:

pDEST11

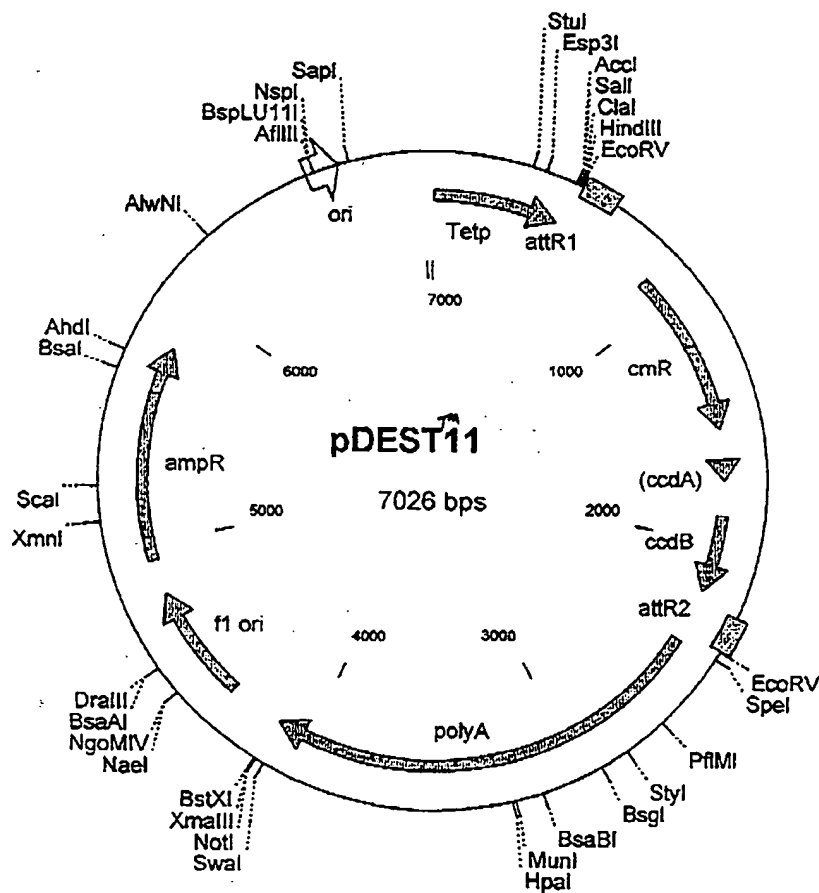
Tet-regulated eukaryotic expression

358 tag tga acc g ^{mRNA from CMV promoter (controlled by tetracycline)} aga tgc cct gga gac gcc atc cac gct gtt ttg acc tcc
 atc act tgg cag tct agc gga cct ctg cgg tag gtg cga caa aac tgg agg

409 ata gaa gac acc ggg acc gat cca gcc tcc gcg gcc ccg aat tgc agc tgc
 tat ctt ctg tgg ccc tgg cta ggt cgg agc cgc cgg ggc tta agc tgc agc

460 gta ccc ggg gat cct cta gag tgc agg ^{Sal} tgc acg gta ^{Clp} ata ^{Hind3} agc ^{EcoRV} ttg ata
 cat ggg ccc cta gga gat ctc agc tcc agc tgc cat agg tat tgc acg tat

511 tca aca agt tgc ~~gag~~ ~~gag~~ ~~gag~~ ~~gag~~ gct gaa cga gaa acg taa dat gat ata aat
 agt tgt tca aac atg ttt ~~tct~~ ~~cga~~ ~~ctt~~ ~~gct~~ ~~cta~~ ~~tgc~~ ~~att~~ ~~tta~~ ~~cta~~ ~~cat~~ ~~tta~~



pDEST11 7026 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
4...479		Tetp ((Tet operator) 7 and min hCMV promoter)
638...514		attR1
888...1547		CmR
1667...1751		inactivated ccdA
1889...2194		ccdB
2235...2359		attR2
2402...4132		polyA
4347...4803		f1 ori
4940...5797		ampR

1	CGAGTTTACC	ACTCCCTATC	AGTGATAGAG	AAAAGTGAAA	GTCGAGTTTA	CCACTCCCTA
61	TCAGTGATAG	AGAAAAGTGA	AAGTCGAGTT	TACCACTCCC	TATCAGTGAT	AGAGAAAAGT
121	GAAAGTCGAG	TTTACCACTC	CCTATCAGTG	ATAGAGAAAA	GTGAAAGTCG	AGTTTACCAC
181	TCCCTATCAG	TGATAGAGAA	AAGTGAAAGT	CGAGTTTACC	ACTCCCTATC	AGTGATAGAG
241	AAAAGTGAAA	GTCGAGTTTA	CCACTCCCTA	TCAGTGATAG	AGAAAAGTGA	AAGTCGAGCT
301	CGGTACCCGG	GTCGAGTAGG	CGTGTACGGT	GGGAGGCCTA	TATAAGCAGA	GCTCGTTTAG
361	TGAACCGTCA	GATCGCCTGG	AGACGCCATC	CACGCTGTTT	TGACCTCCAT	AGAAGACACC
421	GGGACCGATC	CAGCCTCCGC	GGCCCCGAAT	TCGAGCTCGG	TACCCGGGGA	TCCTCTAGAG
481	TCGAGGTCGA	CGGTATCGAT	AAGCTTGATA	TCAACAAGTT	TGTACAAAAA	AGCTGAACGA
541	GAAACGTAAA	ATGATATAAA	TATCAATATA	TTAAATTAGA	TTTGCATAA	AAAACAGACT
601	ACATAATACT	GTAAAACACA	ACATATCCAG	TCATATGGC	GGCCGCTAAG	TTGGCAGCAT
661	CACCCGACGC	ACTTTGCGCC	GAATAAATAC	CTGTGACGGA	AGATCACTTC	GCAGAATAAA
721	TAAATCCTGG	TGTCCCTGTT	GATACCGGGA	AGCCCTGGGC	CACTTTTGG	CGAAAATGAG
781	ACGTTGATCG	GCACGTAAGA	GGTTCCAAC	TTCACCATAA	TGAAATAAGA	TCACTACCGG
841	GCGTATTTTT	TGAGTTATCG	AGATTTTCAG	GAGCTAAGGA	AGCTAAAATG	GAGAAAAAAA
901	TCCTGGGATA	TACCACCGTT	GATATATCCC	AATGGCATCG	TAAAGAACAT	TTTGAGGCAT
961	TTCAAGTCAGT	TGCTCAATGT	ACCTATAACC	AGACCGTTCA	GCTGGATATT	ACGGCCTTTT
1021	TAAAGACCGT	AAAGAAAAAT	AAGCACAAGT	TTTATCCGGC	CTTTATTTCAC	ATTCTTGCCC
1081	GCCTGATGAA	TGCTCATCCG	GAATTCGGTA	TGGCAATGAA	AGACGGTGAG	CTGGTGATAT
1141	GGGATAGTGT	TCACCCTTGT	TACACCGTTT	TCCATGAGCA	AACTGAAACG	TTTTCATCGC
1201	TCTGGAGTGA	ATACCACGAC	GATTTCCGGC	AGTTTCTACA	CATATATTTCG	CAAGATGTGG
1261	CGTGTACCG	TGAAAACCTG	GCCTATTTCC	CTAAAGGGTT	TATTGAGAAT	ATGTTTTTCG
1321	TCTCAGCCAA	TCCCTGGGTG	AGTTTCACCA	GTTTTGATTT	AAACGTGGCC	AATATGGACA
1381	ACTTCTTCGC	CCCCGTTTTT	ACCATGGGCA	AATATTATAC	GCAAGGCGAC	AAGGTGCTGA
1441	TGCCGCTGGC	GATTCAGGTT	CATCATGCCG	TCTGTGATGG	CTTCCATGTC	GGCAGAATGC
1501	TTAATGAATT	ACAACAGTAC	TGCGATGAGT	GGCAGGGCGG	GGCGTAAAGA	TCTGGATCCG
1561	GCTTACTAAA	AGCCAGATAA	CAGTATGCGT	ATTTGCGCGC	TGATTTTTGC	GGTATAAGAA
1621	TATATACTGA	TATGTATACC	CGAAGTATGT	CAAAAAGAGG	TGTGCTATGA	AGCAGCGTAT
1681	TACAGTGACA	GTTGACAGCG	ACAGCTATCA	GTTGCTCAAG	GCATATATGA	TGTCAATATC
1741	TCCGGTCTGG	TAAGCACAAAC	CATGCAGAAT	GAAGCCCGTC	GTCTGCGTGC	CGAACGCTGG
1801	AAAGCGGAAA	ATCAGGAAGG	GATGGCTGAG	GTCGCCCGGT	TTATTGAAAT	GAACGGCTCT
1861	TTTGCTGACG	AGAACAGGGA	CTGGTGAAAT	GCAGTTTAAG	GTTTACACCT	ATAAAAGAGA
1921	GAGCCGTTAT	CGTCTGTTTG	TGGATGTACA	GAGTGATATT	ATTGACACGC	CCGGGCGACG
1981	GATGGTGATC	CCCCTGGCCA	GTGCACGTCT	GCTGTGAGAT	AAAGTCTCCC	GTGAACTTTA
2041	CCCCGTGGTG	CATATCGGGG	ATGAAAGCTG	GCGCATGATG	ACCACCGATA	TGGCCAGTGT
2101	GCCGGTCTCC	GTTATCGGGG	AAGAAGTGGC	TGATCTCAGC	CACCGCGAAA	ATGACATCAA
2161	AAACGCCATT	AACCTGATGT	TCTGGGGAAT	ATAAATGTCA	GGCTCCCTTA	TACACAGCCA
2221	GTCTGCAGGT	CGACCATAGT	GACTGGATAT	GTTGTGTTTT	ACAGTATTAT	GTAGTCTGTT
2281	TTTTATGCAA	AATCTAATTT	AATATATTGA	TATTTATATC	ATTTTACGTT	TCTCGTTTAC
2341	CTTTCTTGTA	CAAAGTGGTT	GATATCGAAT	TCCTGCAGCC	CGGGGGATCC	ACTAGTTCTA
2401	GAGCACTGCG	ATGAGTGGCA	GGGCGGGGCG	TAATTTTTTT	AAGGCAGTTA	TTGGTGCCCT
2461	TAAACGCCTG	GTGCTACGCC	TGAATAAGTG	ATAATAAGCG	GATGAATGGC	AGAAATTTCG
2521	CGGATCTTTG	TGAAGGAACC	TTACTTCTGT	GGTGTGACAT	AATTGGACAA	ACTACCTACA-

FIGURE 31B

74/240

```

2581 GAGATTTAAA GCTCTAAGGT AAATATAAAA TTTTAAAGTG TATAATGTGT TAAACTACTG
2641 ATTCTAATTG TTTGTGTATT TTAGATTCCA ACCTATGGAA CTGATGAATG GGAGCAGTGG
2701 TGGAATGCCT TTAATGAGGA AAACCTGTTT TGCTCAGAAG AAATGCCATC TAGTGATGAT
2761 GAGGCTACTG CTGACTCTCA ACATTCTACT CCTCCAAAAA AGAAGAGAAA GGTAGAAGAC
2821 CCCAAGGACT TTCCTTCAGA ATTGCTAAGT TTTTGTAGTC ATGCTGTGTT TAGTAATAGA
2881 ACTCTTGCTT GCTTTGCTAT TTACACCACA AAGGAAAAAG CTGCACTGCT ATACAAGAAA
2941 ATTATGGAAA AATATTCTGT AACCTTTATA AGTAGGCATA ACAGTTATAA TCATAACATA
3001 CTGTTTTTTC TTAAGTCCACA CAGGCATAGA GTGTCTGCTA TTAATAACTA TGCTCAAAAA
3061 TTGTGTACCT TTAGCTTTTT AATTTGTAAA GGGGTTAATA AGGAATATTT GATGTATAGT
3121 GCCTTGACTA GAGATCATAA TCAGCCATAC CACATTTGTA GAGGTTTTAC TTGCTTTAAA
3181 AAACCTCCCA CACCTCCCC TGAACCTGAA ACATAAAATG AATGCAATTG TTGTTGTTAA
3241 CTTGTTTATT GCAGCTTATA ATGTTTACAA ATAAAGCAAT AGCATCACA ATTTCACAAA
3301 TAAAGCATT TTTTCACTGC ATTCTAGTTG TGGTTTGTCC AAACCTATCA ATGTATCTTA
3361 TCATGTCTGG ATCCCCAGGA AGCTCCTCTG TGTCTCATA AACCTAACC TCCTCTACTT
3421 GAGAGGACAT TCCAATCATA GGCTGCCAT CCACCCTCTG TGTCTCTCTG TTAATTAGGT
3481 CACTTAACAA AAAGGAAATT GGGTAGGGGT TTTTCACAGA CCGCTTTCTA AGGGTAATTT
3541 TAAAATATCT GGGAAGTCCC TTCCACTGCT GTGTTCCAGA AGTGTGGTA AACAGCCAC
3601 AAATGTCAAC AGCAGAAACA TACAAGCTGT CAGCTTTGCA CAAGGGCCCA ACACCCTGCT
3661 CATCAAGAAG CACTGTGGT GCTGTGTTAG TAATGTGCAA AACAGGAGGC ACATTTTCCC
3721 CACCTGTGTA GGTTCACAAA TATCTAGTGT TTTCATTTT ACTTGATCA GGAACCCAGC
3781 ACTCCACTGG ATAAGCATT TCTTATCCA AAACAGCCTT GTGGTCAGTG TTCATCTGCT
3841 GACTGTCAAC TGTAGCATTT TTTGGGGTTA CAGTTTGAGC AGGATATTTG GTCCTGTAGT
3901 TTGCTAACAC ACCCTGCAGC TCCAAAGGTT CCCCACCAAC AGCAAAAAA TGAAAAATTG
3961 ACCCTTGAAT GGGTTTTCCA GCACCATTT CATGAGTTT TTGTGTCCCT GAATGCAAGT
4021 TTAACATAGC AGTTACCCCA ATAACCTCAG TTTTAACAGT AACAGCTTCC CACATCAAAA
4081 TATTTCCACA GGTAAAGTCC TCATTTAAAT TAGGCAAAGG AATTGCTCTA GAGCGGCCGC
4141 CACCGCGGTG GAGCTCCAAT TCGCCCTATA GTGAGTCGTA TTACGCGCGC TCACTGGCCG
4201 TCGTTTTACA ACCTCGTGAC TGGGAAAACC CTGGCGTTAC CCAACTTAAT CGCCTTGCAG
4261 CACATCCCCC TTTCCGCCAGC TGGCGTAATA CGGAAGAGGC CCGCACCGAT CGCCCTTCCC
4321 AACAGTTGCG CAGCCTGAAT GGCGAATGGG ACGCGCCCTG TAGCGGCGCA TTAAGCGCGG
4381 CGGGTGTGGT GGTACGCGC AGCGTGACCG CTACACTTGC CAGCGCCCTA GCGCCCGCTC
4441 CTTTCGCTTT CTTCCCTTCC TTTCTCGCCA CGTTCCGCGG CTTTCCCGT CAAGTCAAT
4501 ATCGGGGGCT CCCTTTAGGG TTCCGATTTA GTGCTTTACG GCACCTCGAC CCAAAAAAAC
4561 TTGATTAGGG TGATGGTTCA CGTAGTGGGC CATCGCCCTG ATAGACGGT TTTCCGCCCT
4621 TGACGTGGGA GTCCACGTT TTAATAGTG GACTCTTGT CCAACTGGA ACAACTCA
4681 ACCCTATCTC GGTCTATTCT TTTGATTTAT AAGGGATTT GCCGATTCG GCCTATTGGT
4741 TAAAAATGA GCTGATTTAA CAAAAATTTA ACGCGAATTT TAACAAAATA TTAACGCTTA
4801 CAATTTAGGT GGCATTTTTC GGGGAAATGT GCGCGGAACC CCTATTTGTT TATTTTTCTA
4861 AATACATTTCA AATATGTATC CGTCAATGAG ACAATAACCC TGATAAATGC TTCAATAATA
4921 TTGAAAAAGG AAGAGTATGA GTATTCAACA TTTCCGTGTC GCCCTTATTC CCTTTTGTG
4981 GGCATTTTGC CTTCTGTGTT TTGCTCACCC AGAAACGCTG GTGAAAGTAA AAGATGCTGA
5041 AGATCAGTTG GGTGCACGAG TGGGTTACAT CGAACTGGAT CTCAACAGCG GTAAGATCCT
5101 TGAGAGTTTT CGCCCCGAAG AACGTTTTCC AATGATGAGC ACTTTTAAAG TTCTGCTATG
5161 TGGCGCGGTA TTATCCCGTA TTGACGCCG GCAAGAGCAA CTCGGTCGCC GCATACACTA
5221 TTCTCAGAA GACTTGGTTG AGTACTCACC AGTCACAGAA AAGCATCTTA CGGATGGCAT
5281 GACAGTAAGA GAATTATGCA GTGCTGCCAT AACCATGAGT GATAACACTG CGGCCAACTT
5341 ACTTCTGACA ACGATCGGAG GACCGAAGGA GCTAACCGCT TTTTTCACAA ACATGGGGGA
5401 TCATGTAACT CGCCTTGATC GTTGGGAACC GGAGCTGAAT GAAGCCATAC CAAACGACGA
5461 GCGTGACACC ACGATGCCTG TAGCAATGGC AACAACGTTG CGCAAACTAT TAACTGGCGA
5521 ACTACTTACT CTAGCTTCCC GGCAACAATT AATAGACTGG ATGGAGGCGG ATAAAGTTGC
5581 AGGACCACTT CTGCGCTCGG CCTTCCGGC TGGCTGTTT ATTGCTGATA AATCTGGAGC
5641 CCGTGAGCGT GGTCTCGCG GTATCATTGC AGCACTGGG CCAGATGGTA AGCCCTCCCG
5701 TATCGTAGTT ATCTACACGA CGGGGAGTCA GGCAACTATG GATGAACGAA ATAGACAGAT
5761 CACTGAGATA GGTGCCTCAC TGATTAAGCA TTGGTAACTG TCAGACCAAG TTTACTCATA
5821 TATAGCTTAG ATTGATTTAA AACTTCATT TTAATTTAAA AGGATCTAGG TGAAGATCCT
5881 TTTTGATAAT CTCATGACCA AAATCCCTTA ACGTGAGTTT TCGTTCCACT GAGCGTCAGA
5941 CCCCCTAGAA AAGATCAAAG GATCTTCTTG AGATCCTTTT TTTCTGCGCG TAATCTGCTG
6001 CTTGCAAACA AAAAAACCAC CGCTACCAGC GGTGGTTTGT TTGCCGGATC AAGAGCTACC-

```

FIGURE 31C

75/240

6061 AACTCTTTTT CCGAAGGTAA CTGGCTTCAG CAGAGCGCAG ATACCAAATA CTGTCCTTCT
6121 AGTGTAGCCG TAGTTAGGCC ACCACTTCAA GAACTCTGTA GCACCGCCTA CATACTCGC
6181 TCTGCTAATC CTGTTACCAG TGGCTGCTGC CAGTGGCGAT AAGTCGTGTC TTACCGGGTT
6241 GGAACAAGG CAGATAGTTAC CGGATAAGGC GCAGCGGTCG GGCTGAACGG GGGGTTTCGTG
6301 CACACAGCCC AGCTTGGAGC GAACGACCTA CACCGAAGT AGATACCTAC AGCGTGAGCT
6361 ATGAGAAAGC GCCACGCTTC CCGAAGGGAG AAAGGCGGAC AGGTATCCGG TAAGCGGCAG
6421 GGTCCGGAACA GGAGAGCGCA CGAGGGAGCT TCCAGGGGGA AACGCCTGGT ATCTTTATAG
6481 TCCTGTCGGG TTTCGCCACC TCTGACTTGA GCGTCGATTT TTGTGATGCT CGTCAGGGGG
6541 GCGGAGCCTA TGGAAAAACG CCAGCAACGC GGCCTTTTTA CGGTTCCCTG CTTTTTGCTG
6601 GCCTTTTGCT CACATGTTCT TTCCTGCGTT ATCCCCTGAT TCTGTGGATA ACCGTATTAC
6661 CGCCTTTGAG TGAGCTGATA CCGCTCGCCG CAGCCGAACG ACCGAGCGCA GCGAGTCAGT
6721 GAGCGAGGAA GCGGAAGAGC GCCCAATACG CAAACCGCCT CTCCCCGCGC GTTGGCCGAT
6781 TCATTAATGC AGCTGGCACG ACAGGTTTCC CGACTGGAAA GCGGGCAGTG AGCGCAACGC
6841 AATTAATGTG AGTTAGCTCA CTCATTAGGC ACCCCAGGCT TTACACTTTA TGCTTCCGGC
6901 TCGTATGTTG TGTGGAATTG TGAGCGGATA ACAATTTTAC ACAGGAAACA GCTATGACCA
6961 TGATTACGCC AAGCGCGCAA TTAACCCTCA CTAAAGGGAA CAAAAGCTGG GTACCGGGCC
7021 CCCCCT

FIGURE 31D

76/240

Figure 32A: pDEST12.2 CMV Promoter for Eukaryotic Expression, SV40 Promoter/ori for G418 Resistance

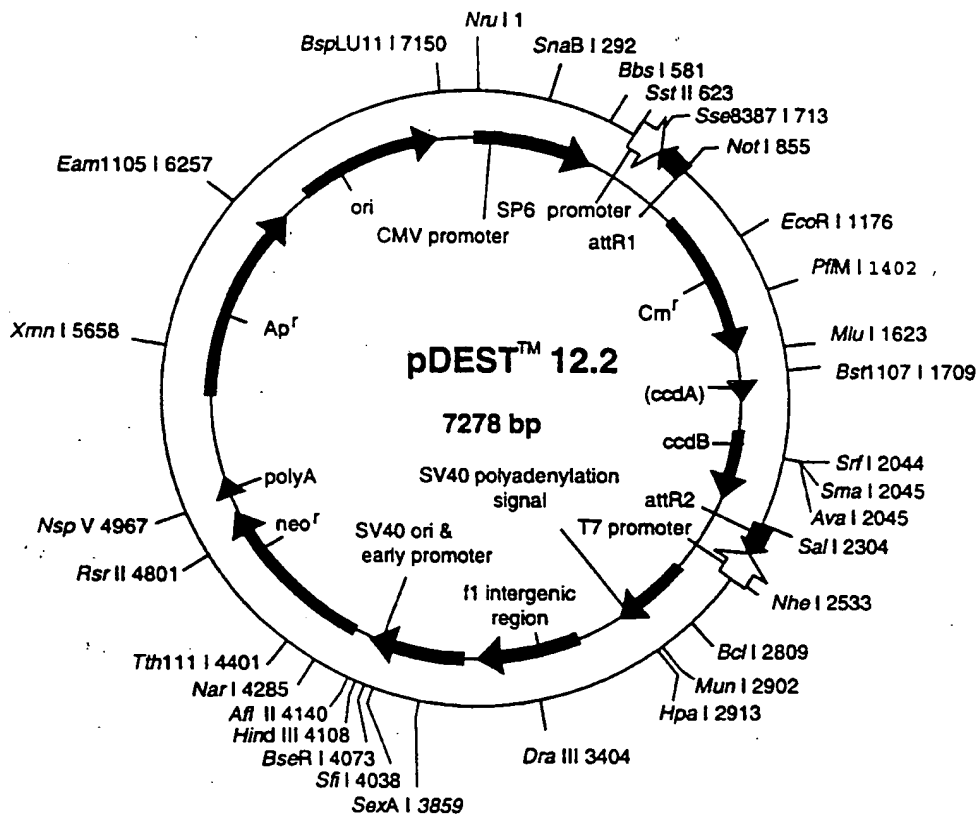
307 *→ mRNA from CMV promoter*
 acc gtc aga tcg cct gga gac gcc atc cac gct gtt ttg acc tcc ata gaa
 tgg cag tct agc gga cct ctg cgg tag gtg cga caa aac tgg agg tat ctt

358 gac acc ggg acc gat cca gcc tcc gga ctc tag cct agg ccg cgg agc gga
 ctg tgg ccc tgg cta ggt cgg agg cct gag atc gga tcc ggc gcc tcg cct

409 taa caa ttt cac aca gga aac agc tat gac cat tag gcc ttt gca aaa agc
 att gtt aaa gtg tgt cct ttg tcg ata ctg gta atc cgg aaa cgt ttt tcg

460 tat tta ggt gac act ata gaa ggt acg cct gca ggt acc ggt ccg gaa ttc
 ata aat cca ctg tga tat ctt cca tgc gga cgt cca tgg cca ggc ctt aag
Age *EcoRI*

511 cca tca aca agt ttg taa ada ada gct gaa cga gaa acg taa aat gat ata
 ggt agt tgt tca aac atg ttg ttt ttt cga cgt gct ctt tgc att gta cca tat
Int *attR1*



77/240

pDEST12.2 7278 bp (rotated to position 3900)

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
86..136		ori
220..742		CMV promoter
1059..935		attR1
1168..1827		CmR
1947..2031		inactivated ccdA
2169..2474		ccdB
2515..2639		attR2
2824..3186		small t & polyA
3310..3378		lac
4363..5157		neo
5680..6540		ampR

1	GGGGGGCGGA	GCCTATGGAA	AAACGCCAGC	AACGCGGCCT	TTTTACGGTT	CCTGGCCTTT
61	TGCTGGCCTT	TTGCTCACAT	GTTCTTTCCT	GCGTTATCCC	CTGATTCTGT	GGATAACCGT
121	ATTACCGCCT	TTGAGTGAGC	TGATACCGCT	CGCCGCAGCC	GAACGACCGA	GCGCAGCGAG
181	TCAGTGAGCG	AGGAAGCGGA	AGAGCTCGCG	AATGCATGTC	GTTACATAAC	TTACGGTAAA
241	TGGCCCGCCT	GGCTGACCGC	CCAACGACCC	CCGCCCATTG	ACGTCAATAA	TGACGTATGT
301	TCCCATAGTA	ACGCCAATAG	GGACTTTCCA	TTGACGTCAA	TGGGTGGAGT	ATTTACGGTA
361	AACTGCCCCAC	TTGGCAGTAC	ATCAAGTGTA	TCATATGCCA	AGTACGCCCC	CTATTGACGT
421	CAATGACGGT	AAATGGCCCG	CCTGGCATT	TGCCCAGTAC	ATGACCTTAT	GGGACTTTCC
481	TACTTGGCAG	TACATCTACG	TATTAGTCAT	CGCTATTACC	ATGGTGATGC	GGTTTTGGCA
541	GTACATCAAT	GGGCGTGGAT	AGCGGTTTGA	CTCACGGGGA	TTTCCAAGTC	TCCACCCCAT
601	TGACGTCAAT	GGGAGTTTGT	TTTGGCACCA	AAATCAACGG	GACTTTCCAA	AATGTCGTAA
661	CAACTCCGCT	CCATTGACGC	AAATGGGCGG	TAGGCGTGTA	CGGTGGGAGG	TCTATATAAG
721	CAGAGCTCGT	TTAGTGAAAC	GTCAGATCGC	CTGGAGACGC	CATCCACGCT	GTTTTGACCT
781	CCATAGAAGA	CACCGGGACC	GATCCAGCCT	CCGGACTCTA	GCCTAGGCCG	CGGGACGGAT
841	AACAATTTCA	CACAGGAAAC	AGCTATGACC	ATTAGGCCTT	TGCAAAAAGC	TATTTAGGTG
901	ACACTATAGA	AGGTACGCCT	GCAGGTACCG	GATCACAAGT	TTGTACAAAA	AAGCTGAACG
961	AGAAACGTAA	AATGATATAA	ATATCAATAT	ATTAAATTAG	ATTTTGATA	AAAAACAGAC
1021	TACATAATAC	TGTAAAACAC	AACATATCCA	GTCACTATGG	CGGCCGCATT	AGGCACCCCA
1081	GGCTTTACAC	TTTATGCTTC	CGGCTCGTAT	AATGTGTGGA	TTTGTAGTTA	GGATCCGTCG
1141	GAGTTTTCAG	GAGCTAAGGA	AGCTAAAATG	GAGAAAAAAA	TCACTGGATA	TACCACCGTT
1201	GATATATCCC	AATGGCATCG	TAAAGAACAT	TTTGAGGCAT	TTCAAGTCAGT	TGCTCAATGT
1261	ACCTATAACC	AGACCGTTCA	GCTGGATATT	ACGGCCTTTT	TAAAGACCGT	AAAGAAAAAT
1321	AAGCACAAGT	TTTATCCGGC	CTTTATTAC	ATTCTTGCCC	GCCTGATGAA	TGCTCATCCG
1381	GAATTCCGTA	TGGCAATGAA	AGACGGTGAG	CTGGTGATAT	GGGATAGTGT	TCACCCTTGT
1441	TACACCGTTT	TCCATGAGCA	AACTGAAACG	TTTTTCATCGC	TCTGGAGTGA	ATACCACGAC
1501	GATTTCCGGC	AGTTTCTACA	CATATATTCG	CAAGATGTGG	CGTGTTACGG	TGAAAACCTG
1561	GCCTATTTCC	CTAAAGGGTT	TATTGAGAAT	ATGTTTTTCG	TCTCAGCCAA	TCCCTGGGTG
1621	AGTTTCACCA	GTTTTGATT	AAACGTGGCC	AATATGGACA	ACTTCTTCGC	CCCCGTTTTT
1681	ACCATGGGCA	AATATTATAC	GCAAGGCGAC	AAGGTGCTGA	TGCCGCTGGC	GATTCAGGTT
1741	CATCATGCCG	TCTGTGATGG	CTTCCATGTC	GGCAGAATGC	TTAATGAATT	ACAACAGTAC
1801	TGCGATGAGT	GGCAGGGCGG	GGCGTAAACG	CGTGGATCCG	GCTTACTAAA	AGCCAGATAA
1861	CAGTATGCGT	ATTGCGCGCG	TGATTTTTGC	GGTATAAGAA	TATATACTGA	TATGTATACC
1921	CGAAGTATGT	CAAAAAGAGG	TGTGCTATGA	AGCAGCGTAT	TACAGTGACA	GTTGACAGCG
1981	ACAGCTATCA	GTTGCTCAAG	GCATATATGA	TGTCAATATC	TCCGGTCTGG	TAAGCACAAC
2041	CATGCAGAAT	GAAGCCCGTC	GTCTGCGTGC	CGAACGCTGG	AAAGCGGAAA	ATCAGGAAGG
2101	GATGGCTGAG	GTGCCCCGGT	TTATTGAAAT	GAACGGCTCT	TTTGCTGACG	AGAACAGGGA
2161	CTGGTGAAAT	GCAGTTTAAG	GTTTACACCT	ATAAAAGAGA	GAGCCGTTAT	CGTCTGTTTG
2221	TGGATGTACA	GAGTGATATT	ATTGACACGC	CCGGGCGACG	GATGGTGATC	CCCCTGGCCA
2281	GTGCACGTCT	GCTGTCAGAT	AAAGTCTCCC	GTGAACTTTA	CCCGGTGGTG	CATATCGGGG
2341	ATGAAAGCTG	GCGCATGATG	ACCACCGATA	TGGCCAGTGT	GCCGGTCTCC	GTTATCGGGG
2401	AAGAAGTGGC	TGATCTCAGC	CACCGCGAAA	ATGACATCAA	AAACGCCATT	AACCTGATGT-

FIGURE 32B

78/240

2461 TCTGGGGAAT ATAAATGTCA GGCTCCCTTA TACACAGCCA GTCTGCAGGT CGACCATAGT
2521 GACTGGATAT GTTGTGTTTT ACAGTATTAT GTAGTCTGTT TTTTATGCAA AATCTAATTT
2581 AATATATTGA TATTTATATC ATTTTACGTT TCTCGTTCAG CTTTCTTGTA CAAAGTGGTG
2641 ATCGCGTGCA TGCGACGTCA TAGCTCTCTC CCTATAGTGA GTCGTATTAT AAGCTAGGCA
2701 CTGGCCGTGCG TTTTACAACG TCGTGACTGG GAAAACCTGCT AGCTTGGGAT CTTTGTGAAG
2761 GAACCTTACT TCTGTGGTGT GACATAATTG GACAAACTAC CTACAGAGAT TTAAAGCTCT
2821 AAGGTAAATA TAAATTTTT AAGTGATATA TGTGTTAAAC TAGCTGCATA TGCTTGTGTC
2881 TTGAGAGTTT TGCTTACTGA GTATGATTTA TGAAATATT ATACACAGGA GCTAGTGATT
2941 CTAATTGTTT GTGTATTTTA GATTCACAGT CCCAAGGCTC ATTTTCAGGCC CCTCAGTCTT
3001 CACAGTCTGT TCATGATCAT AATCAGCCAT ACCACATTG TAGAGGTTT ACTTGCTTTA
3061 AAAAACCTCC CACACCTCCC CCTGAACCTG AAACATAAAA TGAATGCAAT TGTGTGTGTT
3121 AACTTGTTTA TTGCAGCTTA TAATGGTTAC AAATAAAGCA ATAGCATCAC AAATTTTACA
3181 AATAAAGCAT TTTTTTCACT GCATTCTAGT TGTGGTTTGT CCAAACCTCAT CAATGTATCT
3241 TATCATGTCT GGATCGATCC TGCATTAATG AATCGGCCAA CGCGCGGGGA GAGGCGGTTT
3301 GCGTATTGGC TGCGTAATA GCGAAGAGGC CCGCACCGAT CGCCCTTCCC AACAGTTGCG
3361 CAGCCTGAAT GGCGAATGGG ACGCGCCCTG TAGCGGCGCA TTAAGCGCGG CGGGTGTGGT
3421 GGTTACGCGC AGCGTGACCG CTACACTTGC CAGCGCCCTA GCGCCCGCTC CTTTCGCTTT
3481 CTTCCCTTCC TTTCTCGCCA CGTTCGCGCG CTTTCCCGGT CAAGCTCTAA ATCGGGGGCT
3541 CCCTTTAGGG TTCCGATTTA GTGCTTACG GCACCTCGAC CCCAAAAAAC TTGATTAGGG
3601 TGATGGTTCA CGTAGTGGGC CATCGCCCTG ATAGACGGTT TTTCGCCCTT TGACGTTGGA
3661 GTCCACGTTT TTTAATAGTG GACTCTTGT CCAAACCTGA ACAACACTCA ACCCTATCTC
3721 GGTCTATTCT TTTGATTAT AAGGATTTT GCCGATTTCG GCCTATTGGT TAAAAATGA
3781 GCTGATTTAA CAAATATTTA ACGCGAATTT TAACAAAATA TTAACGTTTA CAATTTGCGC
3841 TGATGCGGTA TTTTCTCCTT ACGCATCTGT GCGGTATTTT ACACCGCATA CGCGGATCTG
3901 CGCAGCACCA TGGCCTGAAA TAACCTCTGA AAGAGGAACT TGGTTAGGTA CCTTCTGAGG
3961 CGGAAAGAAC CAGCTGTGGA ATGTGTGTCA GTTAGGGTGT GGAAAGTCCC CAGGCTCCCC
4021 AGCAGGCAGA AGTATGCAAA GCATGCATCT CAATTAGTCA GCAACCAGGT GTGGAAGTCT
4081 CCCAGGCTCC CCAGCAGGCA GAAGTATGCA AAGCATGCAT CTCAATTAGT CAGCAACCAT
4141 AGTCCAGCCC CTAACCTCCG CCATCCCGCC CCTAACCCTG CCCAGTTCCG CCCATTCTCC
4201 GCCCCATGGC TGAATAATTT TTTTATTTA TGCAGAGGCC GAGGCCGCTT CGGCCTCTGA
4261 GCTATTCCAG AAGTAGTGAG GAGGCTTTTT TGGAGGCCTA GGCTTTTGCA AAAAGCTTGA
4321 TTCTTCTGAC ACAACAGTCT CGAACTTAAG GCTAGAGCCA CCATGATTGA ACAAGATGGA
4381 TTGCACGCAG GTTCTCCGGC CGCTTGGGTG GAGAGGCTAT TCGGCTATGA CTGGGCACAA
4441 CAGACAATCG GCTGCTCTGA TGCCCGCGTG TTCCGGCTGT CAGCGCAGGG GCGCCCGGTT
4501 CTTTTTGTCA AGACCGACCT GTCCGGTGCC CTGAATGAAC TGCAGGACGA GGCAGCGCGG
4561 CTATCGTGGC TGGCCACGAC GGGCGTTTCT TGCGCAGCTG TGCTCGACGT TGCTACTGAA
4621 GCGGGAAGGG ACTGGCTGCT ATTGGGCGAA GTGCCGGGGC AGGATCTCCT GTCATCTCAC
4681 CTTGCTCCTG CCGAGAAAGT ATCCATCATG GCTGATGCAA TGCGGCGGCT GCATACGCTT
4741 GATCCGGCTA CCTGCCCAT CGACCACCAA GCGAAACATC GCATCGAGCG AGCACGTACT
4801 CGGATGGAAG CCGGTCTTGT CGATCAGGAT GATCTGGACG AAGAGCATCA GGGGCTCGCG
4861 CCAGCCGAAC TGTTCCGCCAG GCTCAAGGCG CGCATGCCCG ACGGCGAGGA TCTCGTCGTG
4921 ACCCATGGCG ATGCCCTGCTT GCCGAATATC ATGGTGAAA ATGGCCGCTT TTCTGGATTCT
4981 ATCGACTGTG GCCGGCTGGG TGTGGCGGAC CGCTATCAGG ACATAGCGTT GGCTACCCGT
5041 GATATTGCTG AAGAGCTTGG CGGCGAATGG GCTGACCGCT TCCTCGTGCT TTACGGTATC
5101 GCCGCTCCCG ATTCCGACGG CATCGCCTT TATCGCCTT TTGACGAGTT CTTCTGAGCG
5161 GGACTCTGGG GTTCGAAATG ACCGACCAAG CGACGCCCAA CCTGCCATCA CGATGGCCGC
5221 AATAAAATAT CTTTATTTT ATTACATCTG TGTGTTGGTT TTTTGTGTGA ATCGATAGCG
5281 ATAAGGATCC GCGTATGGTG CACTCTCAGT ACAATCTGCT CTGATGCCGC ATAGTTAAGC
5341 CAGCCCCGAC ACCCGCCAAC ACCCGCTGAC GCGCCCTGAC GGGCTTGTCT GCTCCCGGCA
5401 TCCGCTTACA GACAAGCTGT GACCGTCTCC GGGAGCTGCA TGTGTCAGAG GTTTTACCCG
5461 TCATCACCAG AACGCGCGAG ACGAAAGGGC CTCGTGATAC GCCTATTTT ATAGGTTAAT
5521 GTCATGATAA TAATGGTTT TTAGACGTCA GGTGGCACTT TTCGGGGAAA TGTGCGCGGA
5581 ACCCCTATT GTTTATTTT CTAATACAT TCAAATATGT ATCCGCTCAT GAGACAATAA
5641 CCCTGATAAA TGCTTCAATA ATATTGAAAA AGGAAGAGTA TGAGTATTCA ACATTTCCGT
5701 GTCGCCCTTA TTCCCTTTTT TGCGGCATTT TGCTTCTCTG TTTTGTCTCA CCCAGAAACG
5761 CTGGTGAAAG TAAAGATGC TGAAGATCAG TTGGGTGCAC GAGTGGGTTA CATCGAACTG
5821 GATCTCAACA GCGGTAAGAT CCTTGAGAGT TTTGCCCCG AAGAAGCTT TCCAATGATG
5881 AGCACTTTTA AAGTTCTGCT ATGTGGCGCG GTATTATCCC GTATTGACGC CGGGCAAGAG-

FIGURE 32C

79/240

5941 CAACTCGGTC GCCGCATACA CTATTCTCAG AATGACTTGG TTGAGTACTC ACCAGTCACA
6001 GAAAAGCATC TTACGGATGG CATGACAGTA AGAGAATTAT GCAGTGCTGC CATAACCATG
6061 AGTGATAACA CTGCGGCCAA CTTACTTCTG ACAACGATCG GAGGACCGAA GGAGCTAACC
6121 GCTTTTTTGC ACAACATGGG GGATCATGTA ACTCGCCTTG ATCGTTGGGA ACCGGAGCTG
6181 AATGAAGCCA TACCAAACGA CGAGCGTGAC ACCACGATGC CTGTAGCAAT GGCAACAACG
6241 TTGCGCAAAC TATTAAGTGG CGAACTACTT ACTCTAGCTT CCCGGCAACA ATTAATAGAC
6301 TGGATGGAGG CGGATAAAGT TGCAGGACCA CTTCTGCGCT CGGCCCTTCC GGCTGGCTGG
6361 TTTATTGCTG ATAAATCTGG AGCCGGTGAG CGTGGGTCTC GCGGTATCAT TGCAGCACTG
6421 GGGCCAGATG GTAAGCCCTC CCGTATCGTA GTTATCTACA CGACGGGGAG TCAGGCAACT
6481 ATGGATGAAC GAAATAGACA GATCGCTGAG ATAGGTGCCT CACTGATTAA GCATTGGTAA
6541 CTGTCAGACC AAGTTTACTC ATATATACTT TAGATTGATT TAAAACTTCA TTTTAAATTT
6601 AAAAGGATCT AGGTGAAGAT CCTTTTTGAT AATCTCATGA CCAAAATCCC TTAACGTGAG
6661 TTTTCGTTC ACTGAGCGTC AGACCCCGTA GAAAAGATCA AAGGATCTTC TTGAGATCCT
6721 TTTTTTCTGC GCGTAATCTG CTGCTTGCAA ACAAAAAAAC CACCGCTACC AGCGGTGGTT
6781 TGTTTGCCGG ATCAAGAGCT ACCAACTCTT TTTCCGAAGG TAACTGGCTT CAGCAGAGCG
6841 CAGATACCAA ATACTGTCCT TCTAGTGTAG CCGTAGTTAG GCCACCACTT CAAGAAGTCT
6901 GTAGCACCGC CTACATACCT CGCTCTGCTA ATCCTGTTAC CAGTGGCTGC TGCCAGTGGC
6961 GATAAGTCGT GTCTTACCGG GTTGGACTCA AGACGATAGT TACCGGATAA GGCGCAGCGG
7021 TCGGGCTGAA CGGGGGGTTT GTGCACACAG CCCAGCTTGG AGCGAACGAC CTACACCGAA
7081 CTGAGATACC TACAGCGTGA GCATTGAGAA AGCGCCACGC TTCCCGAAGG GAGAAAGGCG
7141 GACAGGTATC CGGTAAGCGG CAGGGTCGGA ACAGGAGAGC GCACGAGGGA GCTTCCAGGG
7201 GGAAACGCCT GGTATCTTTA TAGTCCTGTC GGGTTTCGCC ACCTCTGACT TGAGCGTCGA
7261 TTTTGTGAT GCTCGTCA

FIGURE 32D

80/240

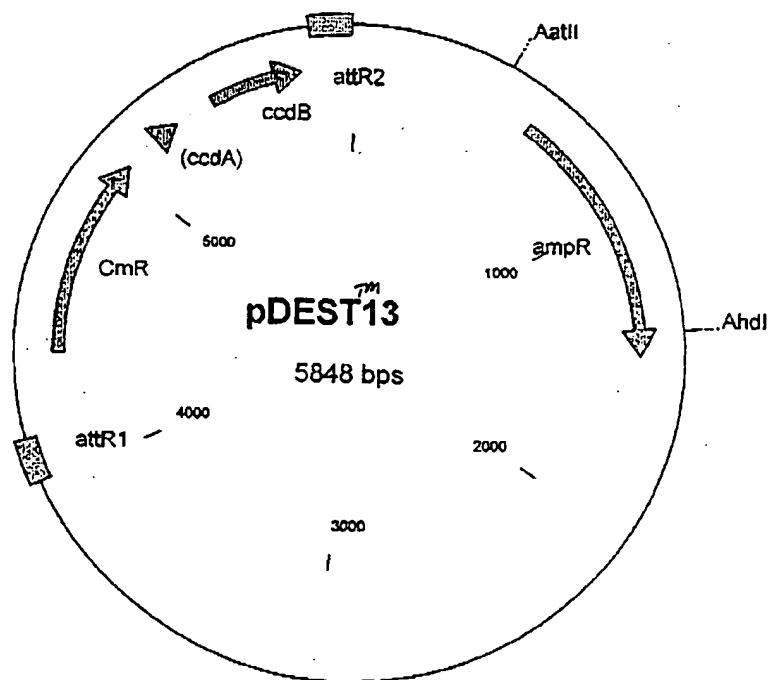
Figure 33A:

pDEST13

Native protein in *E. coli*: λ PL
promoter

3721 tgggcaaacc aagacagcta aagatctctc acctaccaaa caatgcccc ctgcaaaaa
 acccgtttgg ttctgtcgat ttctagagag tggatggttt gttacggggg gacgtttttt
 3781 taaattcata taaaaaacat acagataacc atctgcgggtg ataaattatc tctggcgggtg
 atttaagtat attttttgta tgtctattgg tagacgccac tatttaatag agaccgccac
 3841 ttgacataaa taccactggc ggtgatactg agcacatcag caggacgcac tgaccaccat
aactgtattt atggtgaccg ccactatgac tcgtgtagtc gtctgcgtg actggtggta
 3901 gaaggtgacg ctcttaaaaa ttaagecctg aagaagggca gcattcaaag cagaaggctt
 cttccactgc gagaattttt aattcgggac ttcttccgt cgtaagtttc gtcttccgaa
 3961 tggggtgtgt gatacgaac gaagcattgg gatcatcaca agtttgtaca aaaaagctga
 accccacaca ctatgctttg cttcgtaacc ctagtagtgt tcaaacatgt ttttcgact

BglII
 -35 λ PL Promoter -10
 mRNA
 EcoNI
 Int att R1
 att R2



81/240

pDEST13 5848 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
599..1458		ampR
4123..3998		attR1
4372..5031		CmR
5151..5235		inactivated ccdA
5373..5678		ccdB
5719..5843		attR2
1	T T C A C T G G C C G T C G T T T T A C A A C G T C G T G A C T G G G A A A A C C C T G G C G T T A C C C A A C T T A A	
61	T C G C C T T G C A G C A C A T C C C C C T T T C G C C A G T G G C G A A T A G A G C G A A G A G C C C G C A C C G A	
121	T C G C C C T T C C C A A C A G T T G C G C A G C C T G A A T G G C G A A T G G C G C T G A T G C G G T A T T T T C T	
181	C C T T A C G C A T C T G T G C G T A T T T C A C A C C G C A T A T G G T G C A C T C T C A G T A C A A T C T G C T C	
241	T G A T G C C G C A T A G T T A A G C C A G C C C G A C A C C C G C C A A C A C C C G C T G A C G C G C C T G A C G	
301	G G C T T G T C T G C T C C C G G C A T C C G C T T A C A G A C A A G C T G T G A C C G T C T C C G G G A G C T G C A T	
361	G T G T C A G A G G T T T T C A C C G T C A T C A C C G A A A C G C G C G A G A C G A A A G G G C C T C G T G A T A C G	
421	C C T A T T T T T A T A G G T T A A T G T C A T G A T A A T A A T G G T T T C T T A G A C G T C A G G T G G C A C T T T	
481	T C G G G G A A A T G T G C G C G G A A C C C T A T T T G T T T A T T T T T C T A A A T A C A T T C A A A T A T G T A	
541	T C C G C T C A T G A G A C A A T A A C C C T G A T A A A T G C T T C A A T A A T A T T G A A A A A G G A A G A G T A T	
601	G A G T A T T C A A C A T T T C C G T G T C G C C C T T A T T C C C T T T T T G C G G C A T T T T G C C T T C C T G T	
661	T T T T G C T C A C C A G A A A C G C T G G T G A A A G T A A A A G A T G C T G A A G A T C A G T T G G G T G C A C G	
721	A G T G G G T T A C A T C G A A C T G G A T C T C A A C A G C G G T A A G A T C C T T G A G A G T T T C G C C C C G A	
781	A G A A C G T T T T C C A A T G A T G A G C A C T T T T A A A G T T C T G C T A T G T G G C G C G G T A T T A T C C C G	
841	T A T T G A C G C C G G G C A A G A G C A A C T C G G T C G C C G C A T A C A C T A T T C T C A G A T G A C T T G G T	
901	T G A G T A C T C A C C A G T C A C A G A A A G C A T C T A C G G A T G G C A T G A C A G T A A G A G A A T A T G	
961	C A G T G C T G C C A T A A C C A T G A G T G A T A A C A C T G C G G C C A A C T T A C T T C T G A C A A C C A T C G G	
1021	A G G A C C G A A G G A G C T A A C C G C T T T T T T G C A C A C A T G G G G G A T C A T G T A A C T C G C C T T G A	
1081	T C G T T G G G A A C C G G A G C T G A A T G A A G C C A T A C C A A A C G A C G A G C G T G A C C A C G A T G C C	
1141	T G T A G C A A T G G C A A C A C G T G C G C A A A C T A T T A A C T G G C G A A C T A C T T A C T C T A G C T T C	
1201	C C G G C A A C A A T T A A T A G A C T G G A T G G A G G C G G A T A A A G T T G C A G G A C C A C T T C T G C G C T C	
1261	G G C C C T T C C G G C T G G C T G G T T A T T G C T G A T A A A T C T G G A G C C G G T G A G C G T G G T C T C G	
1321	C G G T A T C A T T G C A G C A C T G G G C C A G A T G G T A A G C C C T C C G T A T C G T A G T T A T C T A C A C	
1381	G A C G G G G A G T C A G G C A A C T A T G G A T G A A C G A A A T A G A C A G A T C G C T G A G A T A G G T G C C T C	
1441	A C T G A T T A A G C A T T G G T A A C T G T C A G A C C A A G T T T A C T C A T A T A C T T T A G A T T G A T T T	
1501	A A A A C T T C A T T T T T A A T T T A A A G G A T C T A G G T G A A G A T C C T T T T T G A T A T C T C A T G A C	
1561	C A A A T C C C T T A A C G T G A G T T T C G T T C C A C T G A G C G T C A G A C C C G T A G A A A A G A T C A A	
1621	A G G A T C T T C T T G A G A T C C T T T T T T C T G C G C G T A A T C T G C T G C T T G C A A A C A A A A A A C C	
1681	A C C G C T A C C A G C G G T G G T T T G T T G C C G G A T C A A G A G C T A C C A A C T C T T T T C C G A A G G T	
1741	A A C T G G C T T C A G C A G A G C G C A G A T A C C A A A T A C T G T T C T T C T A G T G T A G C C G T A G T T A G G	
1801	C C A C C A C T T C A A G A A C T C T G T A G C A C C G C C T A C A T A C C T C G C T C T G C T A A T C C T G T T A C C	
1861	A G T G G C T G C T G C C A G T G G C G A T A A G T C G T G T C T T A C C G G G T T G G A C T C A A G A C G A T A G T T	
1921	A C C G G A T A A G G C G C A G C G G T C G G G C T G A A C G G G G G T T C G T G C A C A C A G C C A G C T T G G A	
1981	G C G A A C G A C C T A C A C C G A A C T G A T A C C T A C A G C G T G A G C A T T G A G A A A G C G C C A C G C T	
2041	T C C C G A A G G G A G A A A G G C G G A C A G G T A T C C G G T A A G C G G C A G G G T C G G A A C A G G A G A G C G	
2101	C A C G A G G G A G C T T C C A G G G G A A A C G C C T G G T A T C T T T A T A G T C C T G T C G G G T T C G C C A	
2161	C C T C T G A C T T G A G C G T C G A T T T T T G T G A T G C T C G T C A G G G G G C G G A G C C T A T G G A A A A A	
2221	C G C C A G C A A C G C G C C T T T T T A C G G T T C C T G G C C T T T T G C T G G C C T T T T G C T C A C A T G T T	
2281	C T T T C C T G C G T T A T C C C C T G A T T C T G T G G A T A A C C G T A T T A C C G C C T T T G A G T G A G C T G A	
2341	T A C C G C T C G C C G C A G C C G A A C G A C C G A G C G C A G C G A G T C A G T G A G C G A G G A A G C G G A A G A	
2401	G C G C C C A A T A C G C A A A C C G C C T C T C C C C G C G C T T G G C C G A T T C A T T A A T G A G A C T G G C A	
2461	C G A C A G G T T T C C C G A C T G G A A A G C G G G C A G T G A G C G C A A C G C A A T T A A T G T A G T T A G C T	
2521	C A C T C A T T A G G C A C C C C A G G C T T T A C A C T T A T G C T T C C G G C T C G T A T G T G T G T G G A A T	
2581	T G T G A G C G G A T A C A A T T T C A C A G G A A A C A G C T A T G A C C A T G A T T A C G C C A A G C T T G G	
2641	C T G C A G G T G A T G A T T A T C A G C C A G C A G A T T A A G G A A A A C A G A C A G G T T A T T G A G C G C	
2701	T T A T C T T T C C C T T A T T T T T G C T G C G G T A A G T C G C A T A A A A A C C A T T C T T C A T A A T T C A A	

FIGURE 33B

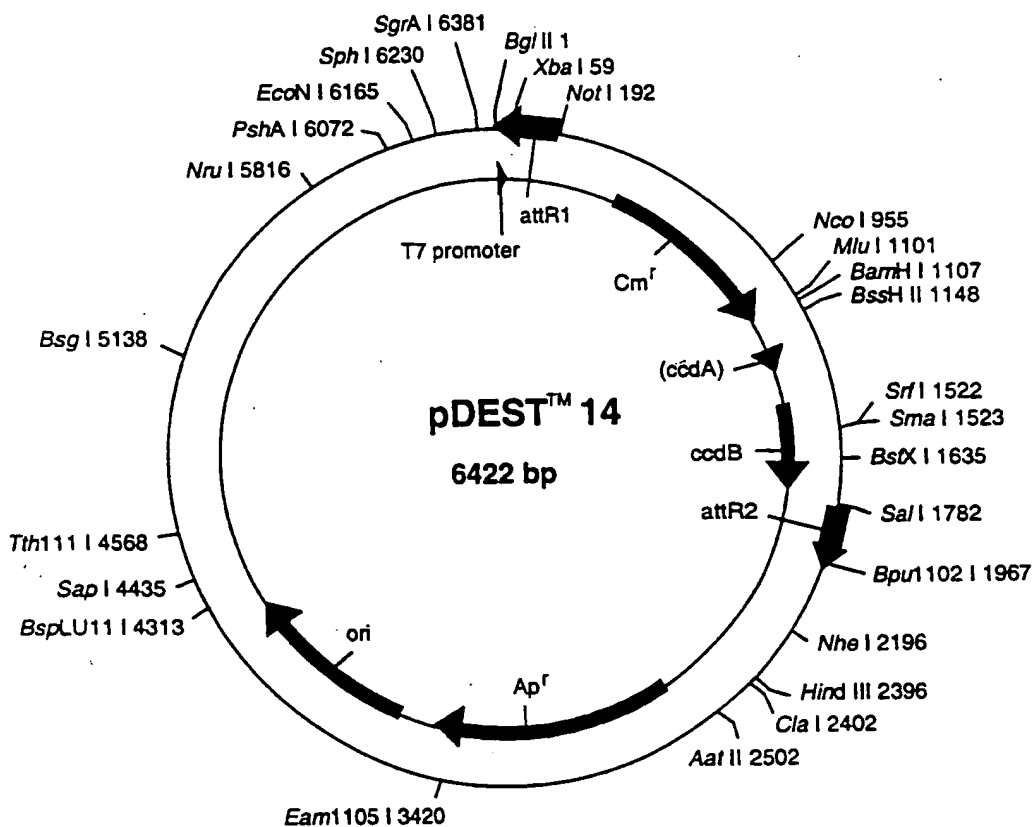
82/240

2761 TCCATTTACT ATGTTATGTT CTGAGGGGAG TGAAAATTCC CCTAATTCTGA TGAAGATTCT
 2821 TGCTCAATTG TTATCAGCTA TGCGCCGACC AGAACACCTT GCCGATCAGC CAAACGTCTC
 2881 TTCAGGCCAC TGACTAGCGA TAACTTTCCC CACAACGGAA CAACTCTCAT TGCATGGGAT
 2941 CATTTGGGTAC TGTGGGTTTA GTGGTTGTAA AAACACCTGA CCGCTATCCC TGATCAGTTT
 3001 CTTGAAGGTA AACTCATCAC CCCCAAGTCT GGCTATGCAG AAATCACCTG GCTCAACAGC
 3061 CTGCTCAGGG TCAACGAGAA TTAACATTCC GTCAGGAAAG CTTGGCTTGG AGCCTGTTGG
 3121 TGCGGTCATG GAATTACCTT CAACCTCAAG CCAGAATGCA GAATCACTGG CTTTTTTGGT
 3181 TGTGCTTACC CATCTCTCCG CATCACCTTT GGTAAAGGTT CTAAGCTTAG GTGAGAACAT
 3241 CCCTGCCTGA ACATGAGAAA AAACAGGGTA CTCATACTCA CTTCTAAGTG ACGGCTGCAT
 3301 ACTAACCGCT TCATACATCT CGTAGATTTT TCTGGCGATT GAAGGGCTAA ATTCTTCAAC
 3361 GCTAACTTTG AGAATTTTTG CAAGCAATGC GCGGTTATAA GCATTTAATG CATTGATGCC
 3421 ATTAAATAAA GCACCAACGC CTGACTGCCC CATCCCCATC TTGCTGCGA CAGATTCTTG
 3481 GGATAAGCCA AGTTTCATTT TTTTCTTTT AATAATTGCT TTAAGGCGAC GTGCGTCTC
 3541 AAGCTGCTCT TGTGTTAATG GTTCTTTTT TGTGCTCATA CGTTAAATCT ATCACCAGCA
 3601 GGGATAAATA TCTAACACCG TCGGTGTTGA CTATTTTACC TCTGGCGGTG ATAATGGTTG
 3661 CATGTACTAA GGAGGTTGTA TGGAAACAAC CATAACCCTG AAAGATTATG CAATGCGCTT
 3721 TGGGCAAACC AAGACAGCTA AAGATCTCTC ACCTACCAAA CAATGCCCCC CTGCAAAAAA
 3781 TAAATTCATA TAAAAACAT ACAGATAACC ATCTGCGGTG ATAAATTATC TCTGGCGGTG
 3841 TTGACATAAA TACCACTGGC GGTGATACTG AGCACATCAG CAGGACGCAC TGACCACCAT
 3901 GAAGGTGACG CTCTTAAAAA TTAAGCCCTG AAGAAGGGCA GCATTCAAAG CAGAAGGCTT
 3961 TGGGGTGTGT GATACGAAAC GAAGCATTGG GATCATCACA AGTTTGTACA AAAAAGCTGA
 4021 ACGAGAAAACG TAAAATGATA TAAATATCAA TATATTAAAT TAGATTTTGC ATAAAAACA
 4081 GACTACATAA TACTGTAAAA CACAACATAT CCAGTCACTA TGGCGGCCGC TAAGTTGGCA
 4141 GCATCACCCG ACGCACTTTG CGCCGAATAA ATACCTGTGA CGGAAGATCA CTTGCGAGAA
 4201 TAAATAAATC CTGGTGTCCC TGTGTATACC GGGAGCCCTT GGGCCAACCT TTGGCGAAAA
 4261 TGAGACGTTG ATCGGCACGT AAGAGTTTCC AACTTTTACC ATAATGAAAT AAGATCACTA
 4321 CCGGGCGTAT TTTTGTAGTT ATCGAGATT TCCAGGAGCTA AGGAAGCTAA AATGGAGAAA
 4381 AAAATCACTG GATATACCAC CGTTGATATA TCCCAATGGC ATCGTAAAGA ACATTTTGAG
 4441 GCATTTTCAGT CAGTTGCTCA ATGTACCTAT AACCAGACCG TTCAGTTGGA TATTACGGCC
 4501 TTTTTAAAGA CCGTAAAGAA AAATAAGCAC AAGTTTATC CGGCCTTTAT TCACATTCTT
 4561 GCCCCCTGA TGAATGCTCA TCCGGAATTC CGTATGGCAA TGAAAGACGG TGAGCTGGTG
 4621 ATATGGGATA GTGTTACCCC TTGTTACACC GTTTTCCATG AGCAAAGTGA AACGTTTTCA
 4681 TCGCTCTGGA GTGAATACCA CGACGATTTC CGGCAGTTTC TACACATATA TTCGCAAGAT
 4741 GTGGCGTGTT ACGGTGAAAA CCTGGCCTAT TTCCCTAAAG GGTATTATGA GAATATGTTT
 4801 TTCGTCTCAG CCAATCCCTG GGTGAGTTTC ACCAGTTTGT ATTTAAACGT GGCCAATATG
 4861 GACAACTTCT TCGCCCCCGT TTTACCATG GGCAAATATT ATACGCAAGG CGACAAGGTG
 4921 CTGATGCCCG TGGCGATTCA GGTTCATCAT GCCGTCTGTG ATGGCTTCCA TGTCGGCAGA
 4981 ATGCTTAATG AATTACAACA GTACTGCGAT GAGTGGCAGG GCGGGGCGTA AACCGGTGGA
 5041 TCCGGCTTAC TAAAAGCCAG ATAACAGTAT GCGTATTTGC GCGCTGATTT TTGCGGTATA
 5101 AGAATATATA CTGATATGTA TACCCGAAGT ATGTCAAAAA GAGGTGTGCT ATGAAGCAGC
 5161 GTATTACAGT GACAGTTGAC AGCGACAGCT ATCAGTTGCT CAAGGCATAT ATGATGTCAA
 5221 TATCTCCGGT CTGGTAAGCA CAACCATGCA GAATGAAGCC CGTCGTCTGC GTGCCGAACG
 5281 CTGGAAAGCG GAAAATCAGG AAGGGATGGC TGAGGTCGCC CGGTTTATTG AAATGAACGG
 5341 CTCTTTTGCT GACGAGAACA GGGACTGGTG AAATGCAGTT TAAGGTTTAC ACCTATAAAA
 5401 GAGAGAGCCG TTATCGTCTG TTTGTGGATG TACAGAGTGA TATTATTGAC ACGCCCGGGC
 5461 GACGGATGGT GATCCCCCTG GCCAGTGCAC GTCTGCTGTC AGATAAAGTC TCCCGTGAAC
 5521 TTTACCCGGT GGTGCATATC GGGGATGAAA GCTGGCGCAT GATGACCACC GATATGGCCA
 5581 GTGTGCCGGT CTCCGTTATC GGGGAAGAAG TGGCTGATCT CAGCCACCGC GAAAATGACA
 5641 TCAAAAACGC CATTAACTG ATGTTCTGGG GAATATAAAT GTCAGGCTCC GTTATACACA
 5701 GCCAGTCTGC AGGTCGACCA TAGTGACTGG ATATGTTGTG TTTTACAGTA TTATGTAGTC
 5761 TGTTTTTTAT GCAAAATCTA ATTTAATATA TTGATATTTA TATCATTTTA CGTTTCTCGT
 5821 TCAGCTTTCT TGTACAAAGT GGTGATAA

FIGURE 33C

Figure 3A: pDEST14 Native Protein Expression in *E. coli*, T7 Promoter

3961 tgcgggcac gatgcgtccg gcgtagagga tcgagatctc gatcccgcca aat^{5'}taatacg^{3'}
 acggccggtg ctacgcaggc cgcattctct agctctagag ctagggcgct ttaattatgc
 4021 // actcactata^{mRNA} gggagaccac aacggtttcc ctctagatca caagtttcta^{attR1} caaaaaagct^{PT7}
 // tgagtgatat ccctctgggtg ttgccaaagg gagatctagt gttcaaacaat gttttttcga //



84/240

pDEST14 6422 bp (rotated to position 4000)

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
185..61		attR1
435..1094		CmR
1214..1298		inactivated ccdA
1436..1741		ccdB
1782..1906		attR2
2632..3489		ampR

1	CGATCCCGCG	AAATTAATAC	GACTCACTAT	AGGGAGACCA	CAACGGTTTC	CCTCTAGATC
61	ACAAGTTTGT	ACAAAAAAGC	TGAACGAGAA	ACGTAAAATG	ATATAAATAT	CAATATATTA
121	AATTAGATTT	TGCATAAAAA	ACAGACTACA	TAATACTGTA	AAACACAACA	TATCCAGTCA
181	CTATGGCGGC	CGCTAAGTTG	GCAGCATCAC	CCGACGCACT	TTGCGCCGAA	TAAATACCTG
241	TGACGGAAGA	TCACTTCGCA	GAATAAATAA	ATCCTGGTGT	CCCTGTTGAT	ACCGGGAAGC
301	CCTGGGCCAA	CTTTTGGCGA	AAATGAGACG	TTGATCGGCA	CGTAAGAGGT	TCCAACTTTC
361	ACCATAATGA	AATAAGATCA	CTACCGGGCG	TATTTTTTGA	GTTATCGAGA	TTTTCAGGAG
421	CTAAGGAAGC	TAAAATGGAG	AAAAAATCA	CTGGATATAC	CACCGTTGAT	ATATCCCAAT
481	GGCATCGTAA	AGAACATTTT	GAGGCATTTT	AGTCAGTTGC	TCAATGTACC	TATAACCAGA
541	CCGTTTCAGT	GGATATTACG	GCCTTTTTAA	AGACCGTAAA	GAAAAATAAG	CACAAGTTTT
601	ATCCGGCCTT	TATTCACATT	CTTGCCCGCC	TGATGAATGC	TCATCCGGAA	TTCCGTATGG
661	CAATGAAAGA	CGGTGAGCTG	GTGATATGGG	ATAGTGTTC	CCCTTGTTAC	ACCGTTTTCC
721	ATGAGCAAAC	TGAAACGTTT	TCATCGCTCT	GGAGTGAATA	CCACGACGAT	TTCCGGCAGT
781	TTCTACACAT	ATATTCGCAA	GATGTGGCGT	GTTACGGTGA	AAACCTGGCC	TATTTCCCTA
841	AAGGGTTTAT	TGAGAATATG	TTTTTCGTCT	CAGCCAATCC	CTGGGTGAGT	TTCAACAGTT
901	TTGATTTAAA	CGTGCCCAAT	ATGGACAAT	TCTTCGCCCC	CGTTTTCAAC	ATGGGCAAA
961	ATTATACGCA	AGGCGACAAG	GTGCTGATGC	CGCTGGCGAT	TCAGGTTTCA	CATGCCGTCT
1021	GTGATGGCTT	CCATGTCCGC	AGAATGCTTA	ATGAATTACA	ACAGTACTGC	GATGAGTGGC
1081	AGGGCGGGGC	GTAAACGCGT	GGATCCGGCT	TACTAAAAGC	CAGATAACAG	TATGCGTATT
1141	TGCGCGCTGA	TTTTTGCGGT	ATAAGAATAT	ATACTGATAT	GTATACCCGA	AGTATGTCAA
1201	AAAGAGGTGT	GCTATGAAGC	AGCGTATTAC	AGTGACAGTT	GACAGCGACA	GCTATCAGTT
1261	GCTCAAGGCA	TATATGATGT	CAATATCTCC	GGTCTGGTAA	GCACAACCAT	GCAGAATGAA
1321	GCCCGTCGTC	TGCGTGCCGA	ACGCTGGAAG	GCGGAAATC	AGGAAGGGAT	GGCTGAGGTC
1381	GCCCGGTTTA	TTGAAATGAA	CGGCTCTTTT	GCTGACGAGA	ACAGGGACTG	GTGAAATGCA
1441	GTTTAAAGTT	TACACCTATA	AAAGAGAGAG	CCGTTATCGT	CTGTTTGTGG	ATGTACAGAG
1501	TGATATTATT	GACACGCCCC	GGCGACGGAT	GGTGATCCCC	CTGGCCAGTG	CACGTCTGCT
1561	GTCAGATAAA	GTCTCCCGTG	AACTTTACCC	GGTGGTGCAT	ATCGGGGATG	AAAGCTGGCG
1621	CATGATGACC	ACCGATATGG	CCAGTGTGCC	GGTCTCCGTT	ATCGGGGAAG	AAGTGGCTGA
1681	TCTCAGCCAC	CGCGAAAATG	ACATCAAAAA	CGCCATTAA	CTGATGTTCT	GGGGAATATA
1741	AATGTCAGGC	TCCCTTATAC	ACAGCCAGTC	TGCAGGTCGA	CCATAGTGAC	TGATATGTG
1801	GTGTTTTTACA	GTATTATGTA	GTCTGTTTTT	TATGCAAAAT	CTAATTTAAT	ATATTGATAT
1861	TTATATCATT	TTACGTTTCT	CGTTCAGCTT	TCTTGTACAA	AGTGGTGATG	ATCCGGCTGC
1921	TAACAAAGCC	CGAAAGGAAG	CTGAGTTGGC	TGCTGCCACC	GCTGAGCAAT	AACTAGCATA
1981	ACCCCTTGGG	GCCTCTAAAC	GGGTCTTGAG	GGGTTTTTTG	CTGAAAGGAG	GAATATATC
2041	CGGATATCCA	CAGGACGGGT	GTGGTCGCCA	TGATCGCGTA	GTCGATAGTG	GCTCCAAGTA
2101	GCGAAGCGAG	CAGGACTGGG	CGGCGGCCAA	AGCGGTCCGA	CAGTGCTCCG	AGAACGGGTG
2161	CGCATAGAAA	TTGCATCAAC	GCATATAGCG	CTAGCAGCAC	GCCATAGTGA	CTGGCGATGC
2221	TGTCGGAATG	GACGATATCC	CGCAAGAGGC	CCGGCAGTAC	CGGCATAACC	AAGCCTATGC
2281	CTACAGCATC	CAGGGTGACG	GTGCCGAGGA	TGACGATGAG	CGCATTGTGA	GATTTTCATC
2341	ACGGTGCCCTG	ACTGCGTTAG	CAATTTAAGT	GTGATAAACT	ACCGCATTAA	AGCTTATCGA
2401	TGATAAGCTG	TCAAACATGA	GAATTCTTGA	AGACGAAAAG	GCCTCGTGAT	ACGCCATTTT
2461	TTATAGGTTA	ATGTCATGAT	AATAATGGTT	TCTTAGACGT	CAGGTGGCAC	TTTTCGGGGA
2521	AATGTGCGCG	GAACCCCTAT	TTGTTTATTT	TTCTAAATAC	ATTCAAATAT	GTATCCGCTC
2581	ATGAGACAA	AACCCTGATA	AATGCTTCAA	TAATATTGAA	AAAGGAAGAG	TATGAGTATT
2641	CAACATTTCC	GTGTCGCCCT	TATTCCTTTT	TTTGCGGCAT	TTTGCTTCC	TGTTTTTGCT
2701	CACCCAGAAA	CGCTGGTGAA	AGTAAAAGAT	GCTGAAGATC	AGTTGGGTGC	ACGAGTGGGT-

Figure 34B

2761 TACATCGAAC TGGATCTCAA CAGCGGTAAG ATCCTTGAGA GTTTTCGCCC CGAAGAACGT
2821 TTTCCAATGA TGAGCACTTT TAAAGTTCTG CTATGTGGCG CGGTATTATC CCGTGTGAC
2881 GCCGGGCAAG AGCAACTCGG TCGCCGCATA CACTATTCTC AGAATGACTT GGTTGAGTAC
2941 TCACCACTCA CAGAAAAGCA TCTTACGGAT GGCATGACAG TAAGAGAATT ATGCAGTGCT
3001 GCCATAACCA TGAGTGATAA CACTGCGGCC AACTTACTTC TGACAACGAT CGGAGGACCG
3061 AAGGAGCTAA CCGCTTTTTT GCACAACATG GGGGATCATG TAACTCGCCT TGATCGTTGG
3121 GAACCGGAGC TGAATGAAGC CATACCAAAC GACGAGCGTG ACACCACGAT GCCTGCAGCA
3181 ATGGCAACAA CGTTGCGCAA ACTATTAAC TGGCGAAGTAC TTACTCTAGC TTCCCGCAA
3241 CAATTAATAG ACTGGATGGA GCGGATAAA GTTGACAGGAC CACTTCTGCG CTCGGCCCTT
3301 CCGGCTGGCT GGTTTATTGC TGATAAATCT GGAGCCGGTG AGCGTGGGTC TCGCGGTATC
3361 ATTGCAGCAC TGGGGCCAGA TGATAAGCCC TCCCGTATCG TAGTTATCTA CACGACGGGG
3421 AGTCAGGCAA CTATGGATGA ACGAAATAGA CAGATCGCTG AGATAGGTGC CTCACTGATT
3481 AAGCATTTGGT AACTGTCAGA CCAAGTTTAC TCATATATAC TTTAGATTGA TTTAAACTTT
3541 CATTTTTTAAT TTTAAAGGAT CTAGGTGAAG ATCCTTTTTG ATAATCTCAT GACCAAAATC
3601 CCTTAACGTG AGTTTTCGTT CCACTGAGCG TCAGACCCCG TAGAAAAGAT CAAAGGATCT
3661 TCTTGAGATC CTTTTTTTCT GCGCGTAATC TGCTGCTTGC AAACAAAAAA ACCACCGCTA
3721 CCAGCGGTGG TTTGTTTGCC GGATCAAGAG CTACCAACTC TTTTCCGAA GGTAACGTGGC
3781 TTCAGCAGAG CGCAGATACC AAATACTGTC CTTCTAGTGT AGCCGTAGTT AGGCCACCAC
3841 TTCAAGAACT CTGTAGCACC GCCTACATAC CTCGCTCTGC TAATCCTGTT ACCAGTGGCT
3901 GCTGCCAGTG GCGATAAGTC GTGTCTTACC GGGTTGGACT CAAGACGATA GTTACCGGAT
3961 AAGGCGCAGC GGTCGGGCTG AACGGGGGGT TCGTGACAC AGCCAGCTT GGAGCGAACG
4021 ACCTACACCG AACTGAGATA CCTACAGCGT GAGCTATGAG AAAGCGCCAC GCTTCCCGAA
4081 GGGAGAAAGG CGGACAGGTA TCCGTAAGC GGCAGGGTCG GAACAGGAGA GCGCAGCAGG
4141 GAGCTTCCAG GGGGAAACGC CTGGTATCTT TATAGTCCTG TCGGGTTTCG CCACCTCTGA
4201 CTTGAGCGTC GATTTTTGTG ATGCTCGTCA GGGGGGCGGA GCCTATGGAA AAACGCCAGC
4261 AACCGGGCCT TTTTACGGTT CTTGGCCTTT TGCTGGCCTT TTGCTCACAT GTTCTTTCTC
4321 CGGTTATCCC CTGATTCTGT GGATAACCGT ATTACCGCCT TTGAGTGAGC TGATACCGCT
4381 CGCCGCGAGC GAACGACCGA GCGCAGCGAG TCAGTGAGCG AGGAAGCGGA AGAGCGCCTG
4441 ATGCGGTATT TTCTCCTTAC GCATCTGTGC GGTATTTTAC ACCGCATATA TGGTGCACCTC
4501 TCAGTACAAT CTGCTCTGAT GCCGCATAGT TAAGCCAGTA TACACTCCGC TATCGCTACG
4561 TGAATGGGTC ATGGCTGCGC CCCGACACCC GCCAACACCC GCTGACGCGC CCTGACGGGC
4621 TTGTCTGCTC CCGGCATCCG CTTACAGACA AGCTGTGACC GTCTCCGGGA GCTGCATGTG
4681 TCAGAGGTTT TCACCGTCAT CACCGAAACG CCGGAGGCAG CTGCGGTAAG GCTCATCAGC
4741 GTGGTCGTGA AGCGATTAC AGATGTCTGC CTGTTTATCC GCGTCCAGCT CGTTGAGTTT
4801 CTCCAGAAGC GTTAATGTCT GGCTTCTGAT AAAGCGGGCC ATGTTAAGGG CGGTTTTTTT
4861 CTGTTTGGTC ACTGATGCCT CCGTGTAAAG GGGATTTCTG TTCATGGGGG TAATGATACC
4921 GATGAAACGA GAGAGGATGC TCACGATACG GGTACTGAT GATGAACATG CCCGTTACT
4981 GGAACGTTGT GAGGGTAAAC AACTGGCGGT ATGGATGCGG CGGGACCAGA GAAAAATCAC
5041 TCAGGGTCAA TGCCAGCGCT TCGTTAATAC AGATGTAGGT GTTCCACAGG GTAGCCAGCA
5101 GCATCCTGCG ATGCAGATCC GGAACATAAT GGTGCAGGGC GCTGACTTCC GCGTTTCCAG
5161 ACTTTACGAA ACACGGAAAC CGAAGACCAT TCATGTTGTT GCTCAGGTCG CAGACGTTTT
5221 GCAGCAGCAG TCGCTTCACG TTCGCTCGCG TATCGGTGAT TCATTCTGCT AACCAAGTAAG
5281 GCAACCCCGC CAGCCTAGCC GGGTCCTCAA CGACAGGAGC ACGATCATGC GCACCCGTGG
5341 CCAGGACCCA ACGCTGCCCG AGATGCGCCG CGTGCGGCTG CTGGAGATGG CGGACGCGAT
5401 GGATATGTTT TGCCAAGGGT TGGTTTGCGC ATTCACAGTT CTCCGCAAGA ATTGATTGGC
5461 TCCAATTCTT GGAGTGGTGA ATCCGTTAGC GAGGTGCCGC CGGCTTCCAT TCAGGTGAG
5521 GTGGCCCGGC TCCATGCACC GCGACGCAAC GCGGGGAGGC AGACAAGGTA TAGGGCGGGC
5581 CCTACAATCC ATGCCAACCC GTTCCATGTG CTCGCCGAGG CGGCATAAAT CGCCGTGACG
5641 ATCAGCGGTC CAGTGATCGA AGTTAGGCTG GTAAGAGCCG CGAGCGATCC TTGAAGCTGT
5701 CCCTGATGGT CGTCATCTAC CTGCTTGGAG AGCATGGCCT GCAACGCGGG CATCCCGATG
5761 CGCCCGGAAG CGAGAAGAAT CATAATGGGG AAGGCCATCC AGCCTCGCGT CGCGAACGCC
5821 AGCAAGACGT AGCCAGCGC GTCGGCCGCC ATGCCGCGCA TAATGGCCTG CTCTCGCCG
5881 AAACGTTTGG TGGCGGGACC AGTGACGAAG GCTTGAGCGA GGGCGTGCAA GATTCCGAAT
5941 ACCGCAAGCG ACAGGCCGAT CATCGTCGCG CTCCAGCGAA AGCGGTCCTC GCCGAAAATG
6001 ACCCAGAGCG CTGCCGCGAC CTGTCTTACG AGTTGCATGA TAAAGAAGAC AGTCATAAGT
6061 GCGGCGACGA TAGTCATGCC CCGCGCCAC CGGAAGGAGC TGAATGGGTT GAAGGCTCTC
6121 AAGGGCATCG GTCGATCGAC GCTCTCCCTT ATGCGACTCC TGCAATAGGA AGCAGCCAG
6181 TAGTAGGTTG AGGCCGTTGA GCACCGCCGC CGCAAGGAAT GGTGCATGCA AGGAGATGGC-

FIGURE 34C

86/240

6241 GCCCAACAGT CCCCCGGCCA CGGGGCCTGC CACCATACCC ACGCCGAAAC AAGCGTCAT
6301 GAGCCCGAAG TGGCGAGCCC GATCTTCCCC ATCGGTGATG TCGGCGATAT AGGCGCCAGC
6361 AACCGCACCT GTGGCGCCGG TGATGCCGGC CACGATGCGT CCGGCGTAGA GGATCGAGAT
6421 CT

FIGURE 34D

Figure 35A: pDEST15 Glutathione-S-transferase Fusion in *E. coli*, T7 Promoter

1 nat cga gat ctc gat ccc gcg aaa tta ata cga ctc act ata ggg aga cca
nta gct cta gag cta ggg cgc ttt aat tat gct gag tga tat ccc tct ggt

52 caa cgg ttt ccc cct aga aat aat ttt gtt taa ctt taa gaa gga gat ata
ggt gcc aaa ggg aga tct tta tta aaa caa att gaa att ctt cct cta tat

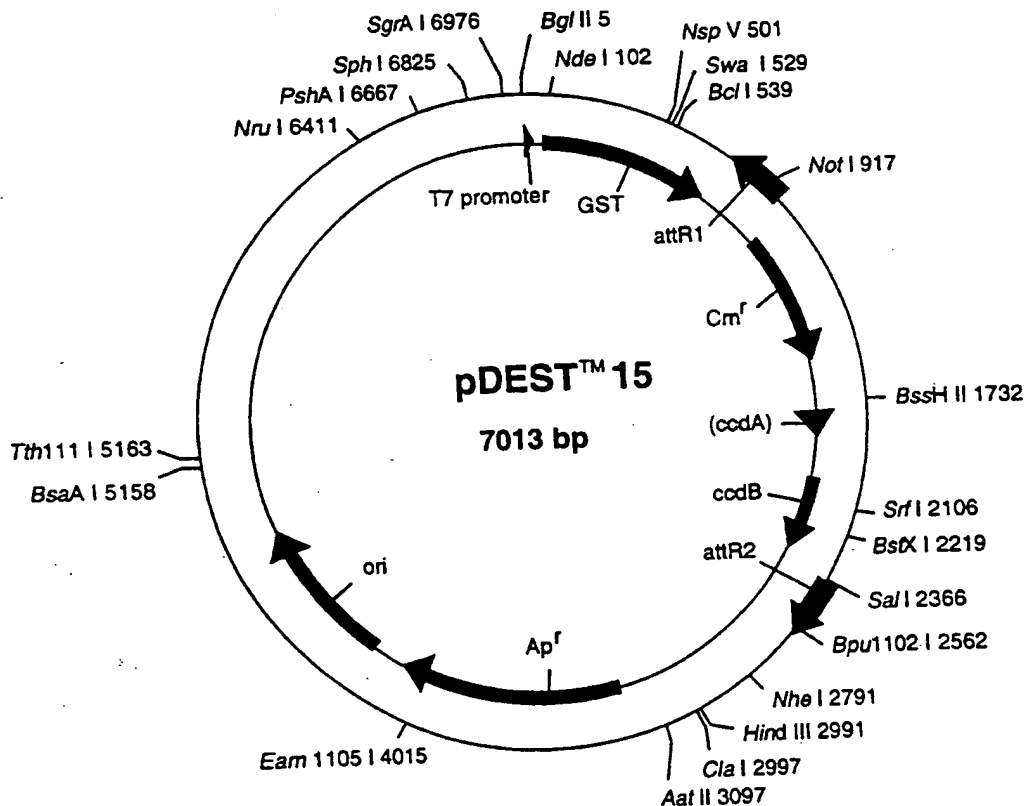
103 *NdeI* *XbaI* *Start Translation* *GST*
cat atg tcc cct ata cta ggt tat tgg aaa att aag ggc ctt gtg caa ccc
gta cac agg gga tat gat cca ata acc ttt taa ttc ccg gaa cac gtt ggg

154 act cga ctt ctt ttg gaa tat ctt gaa gaa aaa tat gaa gag cat ttg tat
tga gct gaa gaa aac ctt ata gaa ctt ctt ttt ata ctt ctc gta aac ata

715 cag ggc tgg caa gcc acg ttt ggt ggt ggc gac cat cct cca aaa tcg gat
gtc ccg acc gtt cgg tgc aaa cca cca ccg ctg gta gga ggt ttt agc cta

766 ctg gtt ccg cgt cca tgg tgg aat caa aca agt tgg tac aaa aaa gct gaa
gac caa ggc gca ggt acc agc tta gtt tgt tca aac atg ttt ttt cga ctt

817 cga gaa acg taa aat gat ata aat atc aat ata tta aat tag att ttg cat
gct ctt tgc att tta cta tat tta tag tta tat aat tta atc taa aac gta



88/240

pDEST15 7013 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
108..776		GST
916..792		attR1
1025..1537		CmR
1804..1888		inactivated ccdA
2026..2331		ccdB
2372..2496		attR2
3233..4093		ampR
1	ATCGAGATCT CGATCCCGCG AAATTAATAC	GACTCACTAT AGGGAGACCA CAACGGTTTC
61	CCTCTAGAAA TAATTTTGTT TAACTTTAAG	AAGGAGATAT ACATATGTCC CCTATACTAG
121	GTTATTGGAA AATTAAGGGC CTTGTGCAAC	CCACTCGACT TCTTTTGGAA TATCTTGAAG
181	AAAAATATGA AGAGCATTTG TATGAGCGCG	ATGAAGGTGA TAAATGGCGA AACAAAAAGT
241	TTGAATTGGG TTTGGAGTTT CCCAATCTTC	CTTATTATAT TGATGGTGAT GTTAAATTAA
301	CACAGTCTAT GGCCATCATA CGTTATATAG	CTGACAAGCA CAACATGTTG GGTGGTTGTC
361	CAAAGAGCG TGCAGAGATT TCAATGCTTG	AAGGAGCGGT TTTGGATATT AGATACGGTG
421	TTTCGAGAAT TGCATATAGT AAAGACTTTG	AAACTCTCAA AGTTGATTTT CTTAGCAAGC
481	TACCTGAAAT GCTGAAAATG TTCGAAGATC	GTTTATGTCA TAAACATAT TTAAATGGTG
541	ATCATGTAAC CCATCCTGAC TTCATGTTGT	ATGACGCTCT TGATGTTGTT TTATACATGG
601	ACCCAATGTG CCTGGATGCG TTCCAAAAT	TAGTTTGTTC TAAAAAACGT ATTGAAGCTA
661	TCCCACAAAT TGATAAGTAC TTGAAATCCA	GCAAGTATAT AGCATGGCCT TTGCAGGGCT
721	GGCAAGCCAC GTTTGGTGGT GCGGACCATC	CTCCAAAATC GGATCTGGTT CCGCGTCCAT
781	GGTCGAATCA AACAAAGTTG TACAAAAAAG	CTGAACGAGA AACGTAAAAT GATATAAATA
841	TCAATATATT AAATTAGATT TTGCATAAAA	AACAGACTAC ATAATACTGT AAAACACAAC
901	ATATCCAGTC ACTATGGCGG CCGCATTAGG	CACCCCAGGC TTTACACTTT ATGCTTCCGG
961	CTCGTATAAT GTGTGGATTT TGAGTTAGGA	TCCGTCGAGA TTTTCAGGAG CTAAGGAAGC
1021	TAAAAATGGAG AAAAAAATCA CTGGATATAC	CACCGTTGAT ATATCCCAAT GGCATCGTAA
1081	AGAACATTTT GAGGCATTTT AGTCAGTTGC	TCAATGTACC TATAACCAGA CCGTTCAGCT
1141	GGATATTACG GCCTTTTAA AGACCGTAAA	GAAAAATAAG CACAAGTTT ATCCGCGCTT
1201	TATTACATT CTTGCCCGCC TGATGAATGC	TCATCCGGAA TTCCGTATGG CAATGAAAGA
1261	CGGTGAGCTG GTGATATGGG ATAGTGTTC	CCCTTGTAC ACCGTTTCC ATGAGCAAAC
1321	TGAAACGTTT TCATCGCTCT GGAGTGAATA	CCACGACGAT TTCCGGCAGT TTCTACACAT
1381	ATATTGCGAA GATGTGGCGT GTTACGGTGA	AAACCTGGCC TATTTCCCTA AAGGGTTTAT
1441	TGAGAAATG TTTTTCGTCT CAGCCAATCC	CTGGGTGAGT TTCACCAGTT TTGATTTAAA
1501	CGTGGCCAAT ATGGACAACT TCTTCGCCCC	CGTTTTACCC ATGGGCAAA ATTATACGCA
1561	AGGCGACAAG GTGCTGATGC CGCTGGCGAT	TCAGGTTTCT CATGCCGTCT GTGATGGCTT
1621	CCATGTCGGC AGAATGCTTA ATGAATTACA	ACAGTACTGC GATGAGTGGC AGGGCGGGGC
1681	GTAATCTAGA GGATCCGGCT TACTAAAAGC	CAGATAACAG TATGCGTATT TGC GCGCTGA
1741	TTTTTGCGGT ATAAGAATAT ATACTGATAT	GTATACCCGA AGTATGTCAA AAAGAGGTGT
1801	GCTATGAAGC AGCGTATTAC AGTGACAGTT	GACAGCGACA GCTATCAGTT GCTCAAGGCA
1861	TATATGATGT CAATATCTCC GGTCTGGTAA	GCACAACCAT GCAGAATGAA GCCCGTCGTC
1921	TGCGTGCCGA ACGCTGAAA GCGGAAAATC	AGGAAGGGAT GGCTGAGGTC GCCCGGTTTA
1981	TTGAAATGAA CGGCTCTTTT GCTGACGAGA	ACAGGGACTG GTGAAATGCA GTTTAAGGTT
2041	TACACCTATA AAAGAGAGAG CCGTTATCGT	CTGTTTGTGG ATGTACAGAG TGATATTATT
2101	GACACGCCCC GCGACGGAT GGTGATCCCC	CTGGCCAGTG CACGTCTGCT GTCAGATAAA
2161	GTCTCCCGTG AACTTTACCC GGTGGTGCAT	ATCGGGGATG AAAGCTGGCG CATGATGACC
2221	ACCGATATGG CCACTGTGCC GGTCTCCGTT	ATCGGGGAAG AAGTGGCTGA TCTCAGCCAC
2281	CGCGAAAATG ACATCAAAAA CGCCATTAAAC	CTGATGTTCT GGGGAATATA AATGTCAGGC
2341	TCCCTTATAC ACAGCCAGTC TGCAGGTGCA	CCATAGTGAC TGGATATGTT GTGTTTACA
2401	GTATTATGTA GTCTGTTTTT TATGCAAAAT	CTAATTTAAT ATATTGATAT TTATATCATT
2461	TTACGTTTCT CGTTCAGCTT TCTTGACAA	AGTGGTTTGA TTCGACCCGG GATCCGGCTG
2521	CTAACAAAGC CCGAAAGGAA GCTGAGTTGG	CTGCTGCCAC CGCTGAGCAA TAACTAGCAT
2581	AACCCCTTGG GGCCTCTAAA CGGGTCTTGA	GGGGTTTTTT GCTGAAAGGA GGAATATAT
2641	CCGATATCC ACAGGACGGG TGTGGTCGCC	ATGATCGCGT AGTCGATAGT GGCTCCAAGT

FIGURE 35B

89/240

```

2701 AGCGAAGCGA GCAGGACTGG GCGGCGGCCA AAGCGGTCGG ACAGTGCTCC GAGAACGGGT
2761 GCGCATAGAA ATTGCATCAA CGCATATAGC GCTAGCAGCA CGCCATAGTG ACTGGCGATG
2821 CTGTCCGAAT GGACGATATC CCGCAAGAGG CCCGGCAGTA CCGGCATAAC CAAGCCTATG
2881 CCTACAGCAT CCAGGGTGAC GGTGCCGAGG ATGACGATGA GCGCATTGTT AGATTTTCATA
2941 CACGGTGCCT GACTGCGTTA GCAATTTAAC TGTGATAAAC TACCGCATT AAGCTTTTCG
3001 ATGATAAGCT GTCAAACATG AGAATTCTTG AAGACGAAAG GGCTCTGTGA TACGCCTATT
3061 TTTATAGGTT AATGTCATGA TAATAATGGT TTCTTAGACG TCAGGTGGCA CTTTTTCGGG
3121 AAATGTGCGC GGAACCCCTA TTTGTTTATT TTTCTAAATA CATTCAAATA TGTATCCGCT
3181 CATGAGACAA TAACCCTGAT AAATGCTTCA ATAATATTGA AAAAGGAAGA GTATGAGTAT
3241 TCAACATTTT CGTGTGCGCC TTATTCCTTT TTTTGCGGCA TTTTGCCCTT CTGTTTTTTC
3301 TCACCCAGAA ACGCTGGTGA AAGTAAAGA TGCTGAAGAT CAGTTGGGTG CACGAGTGGG
3361 TTACATCGAA CTGGATCTCA ACAGCGGTAA GATCCTTGAG AGTTTTTCGCC CCGAAGAACG
3421 TTTTCCAATG ATGAGCACTT TTAAAGTTCT GCTATGTGGC GCGGTATTAT CCGGTGTTGA
3481 CGCCGGGCAA GAGCAACTCG GTCGCCGCAT ACACTATTCT CAGAATGACT TGGTTGAGTA
3541 CTCACCAGTC ACAGAAAAGC ATCTTACGGA TGGCATGACA GTAAGAGAAT TATGCAGTGC
3601 TGCCATAACC ATGAGTGATA ACACTGCGGC CAACTTACTT CTGACAACGA TCGGAGGACC
3661 GAAGGAGCTA ACCGCTTTTT TGCACAACAT GGGGGATCAT GTAACTCGCC TTGATCGTTG
3721 GGAACCGGAG CTGAATGAAG CCATACCAA CGACGAGCGT GACACCACGA TGCCTGCAGC
3781 AATGGCAACA ACGTTGCGCA AACTATTAAC TGGCGAACTA CTTACTCTAG CTTCCCGGCA
3841 ACAATTAATA GACTGGATGG AGGCGGATA AGTTGCAGGA CCACTTCTGC GCTCGGCCCT
3901 TCCGGCTGGC TGGTTTATTG CTGATAAATC TGGAGCCGGT GAGCGTGGGT CTCGCGGTAT
3961 CATTGCAGCA CTGGGGCCAG ATGGTAAGCC CTCCCGTATC GTAGTTATCT ACACGACGGG
4021 GAGTCAGGCA ACTATGGATG AACGAAATAG ACAGATCGCT GAGATAGGTG CCTCACTGAT
4081 TAAGCATTGG TAACTGTCAG ACCAAGTTTA CTCATATATA CTTTAGATTG ATTTAAACT
4141 TCATTTTTAA TTTAAAGGA TCTAGGTGAA GATCCTTTTT GATAATCTCA TGACCAAAAT
4201 CCCTTAACGT GAGTTTTCGT TCCACTGAGC GTCAGACCCC GTAGAAAAGA TCAAAGGATC
4261 TTCTTGAGAT CCTTTTTTTC TGCGCGTAAT CTGCTGCTTG CAAACAAAAA AACCACCGCT
4321 ACCAGCGGTG GTTGTGTTGC CGGATCAAGA GCTACCAACT CTTTTCCGA AGGTAACCTG
4381 CTTTCAGAGA GCGCAGATAC CAAATACTGT CTTCTAGTG TAGCCGTAGT TAGCCACCA
4441 CTTCAAGAAC TCTGTAGCAC CGCCTACATA CCTCGCTCTG CTAATCCTGT TACCAGTGGC
4501 TGCTGCCAGT GGCGATAAGT CGTGTCTTAC CGGGTTGGAC TCAAGACGAT AGTTACCGGA
4561 TAAGGCGCAG CGGTGCGGCT GAACGGGGGG TTCGTGCACA CAGCCCAGCT TGGAGCGAAC
4621 GACCTACACC GAACTGAGAT ACCTACAGCG TGAGCTATGA GAAAGCGCCA CGCTTCCCGA
4681 AGGAGAAAG GCGGACAGGT ATCCGGTAAG CGGCAGGGTC GGAACAGGAG AGCGCACGAG
4741 GGAGCTTCCA GGGGGAACG CCTGGTATCT TTATAGTCCT GTCGGGTTTC GCCACCTCTG
4801 ACTTCAGCGT CGATTTTTGT GATGCTCGTC AGGGGGGCGG AGCCTATGGA AAAACGCCAG
4861 CAACGCGGCC TTTTACGGT TCCTGGCCTT TTGCTGGCCT TTTGCTCACA TGTTCTTCC
4921 TGCGTTATCC CCTGATTCTG TGGATAACCG TATTACCGCC TTTGAGTGAG CTGATACCGC
4981 TCGCCGACG CGAACGACCG AGCGCAGCGA GTCAGTGAGC GAGGAAGCGG AAGAGCGCCT
5041 GATGCGGTAT TTTCTCCTTA CGCATCTGTG CGGTATTTCA CACCGCATAT ATGGTGCAC
5101 CTCAGTACAA TCTGCTCTGA TGCCGCATAG TTAAGCCAGT ATACACTCCG CTATCGCTAC
5161 GTGACTGGGT CATGGCTGCG CCCCGACACC CGCCAACACC CGCTGACCGG CCCTGACGGG
5221 CTTGTCTGCT CCCGGCATCC GCTTACAGAC AAGCTGTGAC CGTCTCCGGG AGCTGCATGT
5281 GTCAGAGGTT TTCACCGTCA TCACCGAAGC GCGCGAGGCA GCTGCGGTAA AGCTCATCAG
5341 CGTGGTCTGT AAGCGATTCA CAGATGCTCG CCTGTTTCATC CGCGTCCAGC TCGTTGAGTT
5401 TCTCCAGAAG CGTTAATGTC TGGCTTCTGA TAAAGCGGGC CATGTTAAGG GCGGTTTTTT
5461 CCTGTTTGGT CACTGATGCC TCCGTGTAAG GGGGATTCT GTTCATGGGG GTAATGATAC
5521 CGATGAAACG AGAGAGGATG CTCACGATAC GGGTTACTGA TGATGAACAT GCCCGGTTAC
5581 TGGAACGTTG TGAGGGTAAA CAACTGGCGG TATGGATGCG GCGGGACCAG AGAAAAATCA
5641 CTCAGGGTCA ATGCCAGCGC TTCGTTAATA CAGATGTAGG TGTTCCACAG GGTAGCCAGC
5701 AGCATCTCTG GATGCAGATC CGGAACATAA TGGTGCAGGG CGCTGACTTC CGCGTTTCCA
5761 GACTTTACGA AACACGGAAC CCGAAGACCA TTCATGTTGT TGCTCAGGTC GCAGACGTTT
5821 TGCAGCAGCA GTCGCTTCAC GTTCGCTCGC GTATCGGTGA TTCATTCTGC TAACCAAGTAA
5881 GGCAACCCCG CCAGCCTAGC CGGGTCTCTA ACGACAGGAG CACGATCATG CGCACCCGTG
5941 GCCAGGACCC AACGCTGCCC GAGATGCGCC GCGTGCGGCT GCTGGAGATG GCGGACGCGA
6001 TGGATATGTT CTGCCAAGGG TTGGTTTTCG CATTACAGT TCTCCGAAG AATTGATTGG
6061 CTCCAATTCT TGGAGTGGTG AATCCGTTAG CGAGGTGCCG CCGCTTCCA TTCAGGTCTGA
6121 GGTGGCCCGG CTCCATGCAC CGCGACGCAA CGCGGGGAGG CAGACAAGGT ATAGGGCGGC

```

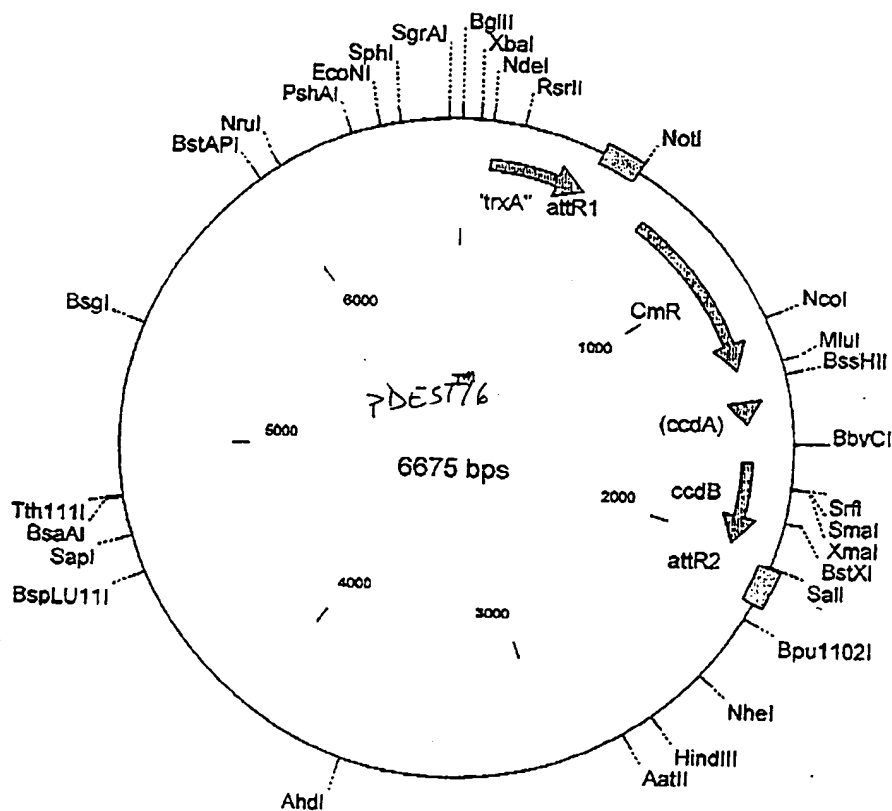
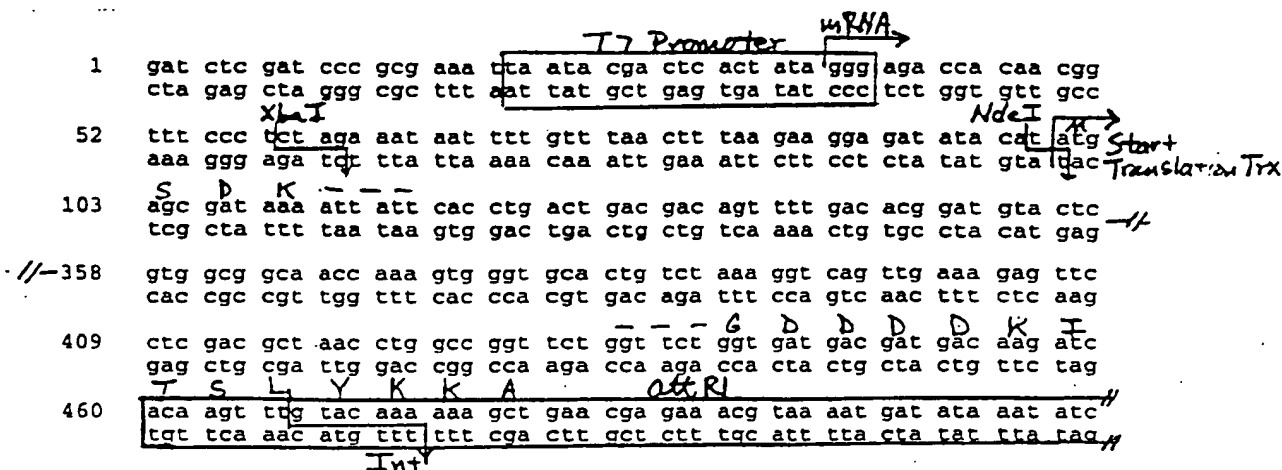
FIGURE 35C

90/240

6181 GCCTACAATC CATGCCAACC CGTTCCATGT GCTCGCCGAG GCGGCATAAA TCGCCGTGAC
6241 GATCAGCGGT CCAGTGATCG AAGTTAGGCT GGTAAGAGCC GCGAGCGATC CTTGAAGCTG
6301 TCCCTGATGG TCGTCATCTA CCTGCCTGGA CAGCATGGCC TGCAACGCGG GCATCCCGAT
6361 GCCGCCGGAA GCGAGAAGAA TCATAATGGG GAAGGCCATC CAGCCTCGCG TCGCGAACGC
6421 CAGCAAGACG TAGCCCAGCG CGTCGGCCGC CATGCCGGCG ATAATGGCCT GCTTCTCGCC
6481 GAAACGTTTG GTGGCGGGAC CAGTGACGAA GGCTTGAGCG AGGGCGTGCA AGATTCCGAA
6541 TACCGCAAGC GACAGGCCGA TCATCGTCGC GCTCCAGCGA AAGCGGTCCT CGCCGAAAA
6601 GACCCAGAGC GCTGCCGGCA CCTGTCCTAC GAGTTGCATG ATAAAGAAGA CAGTCATAAG
6661 TCGGGCGACG ATAGTCATGC CCCGCGCCCA CCGGAAGGAG CTGACTGGGT TGAAGGCTCT
6721 CAAGGGCATC GGTTCGATCGA CGCTCTCCCT TATGCGACTC CTGCATTAGG AAGCAGCCCA
6781 GTAGTAGGTT GAGGCCGTTG AGCACCGCCG CCGCAAGGAA TGGTGCATGC AAGGAGATGG
6841 CGCCCAACAG TCCCCCGGCC ACGGGGCCTG CCACCATACC CACGCCGAAA CAAGCGCTCA
6901 TGAGCCCGAA GTGGCGAGCC CGATCTTCCC CATCGGTGAT GTCGGCGATA TAGGCGCCAG
6961 CAACCGCACC TGTGGCGCCG GTGATGCCGG CCACGATGCG TCCGGCGTAG AGG

FIGURE 351)

91/260

Figure 36A: γ DEST16Thioredoxin N-Fusion Protein
in E. coli with T7 Promoter

92/240

pDEST16 6675 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
104..457		trxA
585..461		attR1
694..1353		CmR
1473..1557		inactivated ccdA
1695..2000		ccdB
2041..2165		attR2
1	AGATCTCGAT CCCGCGAAAT TAATACGACT CACTATAGGG AGACCACAAC GGTTTCCCTC	
61	TAGAAATAAT TTTGTTTAACT TTTAAGAAGG AGATATACAT ATGAGCGATA AAATTATTC	
121	CCTGACTGAC GACAGTTTGG ACACGGATGT ACTCAAAGCG GACGGGGCGA TCCTCGTCCA	
181	TTTCTGGGCA GAGTGGTGCG GTCCGTGCAA AATGATCGCC CCGATTCTGG ATGAAATCGC	
241	TGACGAATAT CAGGGCAAAC TGACCGTTGC AAAACTGAAC ATCGATCAAA ACCCTGGCAC	
301	TGCGCCGAAA TATGGCATCC GTGGTATCCC GACTCTGCTG CTGTTCAAAA ACGGTGAAGT	
361	GGCGGCAACC AAAGTGGGTG CACTGTCTAA AGGTCAGTTG AAAGAGTTCC TCGACGCTAA	
421	CCTGGCCGGT TCTGGTTCTG GTGATGACGA TGACAAGATC ACAAGTTTGT ACAAAAAAGC	
481	TGAACGAGAA ACGTAAAATG ATATAAATAT CAATATATTA AATTAGATTT TGCATAAAAA	
541	ACAGACTACA TAATACTGTA AAACACAACA TATCCAGTCA CTATGGCGGC CGCATTAGGC	
601	ACCCAGGCT TTACACTTTA TGCTTCCGGC TCGTATAATG TGTGGATTTT GAGTTAGGAT	
661	CCGGCGAGAT TTTCAGGAGC TAAGGAAGCT AAAATGGAGA AAAAAATCAC TGGATATACC	
721	ACCGTTGATA TATCCCAATG GCATCGTAAA GAACATTTTG AGGCATTTC	
781	CAATGTACCT ATAACCAGAC CGTTCAGCTG GATATTACGG CCTTTTAA	
841	AAAAATAAGC ACAAGTTTAA TCCGGCCTTT ATTACATTC TTGCCCGCT	
901	CATCCGGAAT TCCGTATGGC AATGAAAGAC GGTGAGCTGG TGATATGGGA TAGTGTTCAC	
961	CCTTGTACCA CCGTTTCCA TGAGCAAAC TAAACGTTT CATCGCTCTG GAGTGAATAC	
1021	CACGACGATT TCCGGCAGTT TCTACACATA TATTCGCAAG ATGTGGCGTG TTACGGTGAA	
1081	AACCTGGCCT ATTTCCCTAA AGGGTTTATT GAGAATATGT TTTTCGTCTC AGCCAATCCC	
1141	TGGGTGAGTT TCACCAAGTT TGATTTAAAC GTGGCCAATA TGGACAACCT CTTCGCCCCC	
1201	GTTTTACCA TGGGCAAATA TTATACGCAA GCGACAAGG TGCTGATGCC GCTGGCGATT	
1261	CAGGTTTCATC ATGCCGTCTG TGATGGCTTC CATGTGCGCA GAATGCTTAA TGAATTACAA	
1321	CAGTACTGCG ATGAGTGGCA GGGCGGGGCG TAAACGCGTG GATCCGGCTT ACTAAAAGCC	
1381	AGATAACAGT ATGCGTATTT GCGCGTGTAT TTTTGCGGTA TAAGAATATA TACTGATATG	
1441	TATACCCGAA GTATGTCAAA AAGAGGTGTG CTATGAAGCA GCGTATTACA GTGACAGTTG	
1501	ACAGCGACAG CTATCAGTTG CTCAAGGCAT ATATGATGTC AATATCTCCG GTCTGGTAA	
1561	CACAACCATG CAGAATGAAG CCCGTCGTCT GCGTGCCGAA CGCTGGAAAG CGGAAAATCA	
1621	GGAAGGGATG GCTGAGGTCG CCGGTTTAT TGAAATGAAC GGCTCTTTTG CTGACGAGAA	
1681	CAGGGACTGG TGAAATGCAG TTAAAGGTTT ACACCTATAA AAGAGAGAGC CGTTATCGTC	
1741	TGTTTGTGGA TGTACAGAGT GATATTATTG ACACGCCCGG GCGACGGATG GTGATCCCCC	
1801	TGGCCAGTGC ACGTCTGCTG TCAGATAAAG TCTCCCGTGA ACTTTACCCG GTGGTGCATA	
1861	TCGGGGATGA AAGCTGGCGC ATGATGACCA CCGATATGGC CAGTGTGCCG GTCTCCGTTA	
1921	TCGGGGAAGA AGTGGCTGAT CTCAGCCACC GCGAAAATGA CATCAAAAAC GCCATTAACC	
1981	TGATGTTCTG GGAATATAA ATGTCAGGCT CCCTTATACA CAGCCAGTCT GCAGGTCGAC	
2041	CATAGTGACT GGATATGTTG TGTTTTACAG TATTATGTAG TCTGTTTTTT ATGCAAAATC	
2101	TAATTTAATA TATTGATATT TATATCATTT TACGTTTCTC GTTCAGCTTT CTTGTACAAA	
2161	GTGGTGATGA TCCGGCTGCT AACAAAGCCC GAAAGGAAGC TGAGTTGGCT GCTGCCACCG	
2221	CTGAGCAATA ACTAGCATAA CCCCTTGGGG CCTCTAAACG GGTCTTGAGG GGTTTTTTGC	
2281	TGAAAGGAGG AACTATATCC GGATATCCAC AGGACGGGTG TGGTCGCCAT GATCGCGTAG	
2341	TCGATAGTGG CTCCAAGTAG CGAAGCGAGC AGGACTGGGC GGCGGCCAAA GCGGTCCGAC	
2401	AGTGCTCCGA GAACGGGTGC GCATAGAAAT TGCATCAACG CATATAGCGC TAGCAGCAGC	
2461	CCATAGTGAC TGGCGATGCT GTCGGAATGG ACGATATCCC GCAAGAGGCC CGGCAGTACC	
2521	GGCATAACCA AGCCTATGCC TACAGCATCC AGGGTGACGG TGCCGAGGAT GACGATGAGC	
2581	GCATTGTTAG ATTTATACA CCGTGCTGTA CTGCGTTAGC AATTAACTG TGATAAACTA	
2641	CCGCATTAAA GCTTATCGAT GATAAGCTGT CAAACATGAG AATTCTTGAA GACGAAAGGG	
2701	CCTCGTGATA CGCCTATTTT TATAGGTTAA TGTCATGATA ATAATGGTTT CTTAGACGTC	
2761	AGGTGGCACT TTTCGGGGAA ATGTGCGCGG AACCCCTATT TGTTTATTTT TCTAAATACA-	

Figure 36B

93/240

2821 TTCAAATATG TATCCGCTCA TGAGACAATA ACCCTGATAA ATGCTTCAAT AATATTGAAA
2881 AAGGAAGAGT ATGAGTATTC AACATTTCCG TGTCGCCCTT ATTCCTTTT TTGCGGCATT
2941 TTGCCTTCCT GTTTTGTCTC ACCCAGAAAC GCTGGTGAAA GTAAAAGATG CTGAAGATCA
3001 GTTGGGTGCA CGAGTGGGTT ACATCGAACT GGATCTCAAC AGCGGTAAGA TCCTTGAGAG
3061 TTTTCGCCCC GAAGAACGTT TTCCAATGAT GAGCACTTTT AAAGTTCTGC TATGTGGCGC
3121 GGTATTATCC CGTGTGACG CCGGGCAAGA GCAACTCGGT CGCCGCATAC ACTATTCTCA
3181 GAATGACTTG GTTGAGTACT CACCAGTCAC AGAAAAGCAT CTTACGGATG GCATGACAGT
3241 AAGAGAATTA TGCAGTGCTG CCATAACCAT GAGTGATAAC ACTGCGGCCA ACTTACTTCT
3301 GACAACGATC GGAGGACCGA AGGAGCTAAC CGCTTTTTTG CACAACATGG GGGATCATGT
3361 AACTCGCCTT GATCGTTGGG AACC GGAGCT GAATGAAGCC ATACCAAACG ACGAGCGTGA
3421 CACCACGATG CCTGCAGCAA TGGCAACAAC GTTGCGCAAA CTATTAACTG GCGAACTACT
3481 TACTCTAGCT TCCCGGCAAC AATTAATAGA CTGGATGGAG GCGGATAAAG TTGCAGGACC
3541 ACTTCTGCGC TCGGCCCTTC CGGCTGGCTG GTTTATTGCT GATAAATCTG GAGCCGGTGA
3601 GCGTGGGTCT CGCGGTATCA TTGCAGCACT GGGGCCAGAT GGTAAGCCCT CCCGTATCGT
3661 AGTTATCTAC ACGACGGGGA GTCAGGCAAC TATGGATGAA CGAAATAGAC AGATCGCTGA
3721 GATAGGTGCC TCACTGATTA AGCATTGGTA ACTGTCAGAC CAAGTTTACT CATATATACT
3781 TTAGATTGAT TTAAAACTTC ATTTTTAATT TAAAAGGATC TAGGTGAAGA TCCTTTTTGA
3841 TAATCTCATG ACCAAAATCC CTTAACGTGA GTTTTCGTTC CACTGAGCGT CAGACCCCGT
3901 AGAAAAGATC AAAGGATCTT CTTGAGATCC TTTTTTCTG CGCGTAATCT GCTGCTTGCA
3961 AACAAAAAAA CCACCGCTAC CAGCGTGGT TTGTTTGCCG GATCAAGAGC TACCAACTCT
4021 TTTTCCGAAG GTAAGTGGT TCAGCAGAGC GCAGATACCA AATACTGTCC TTCTAGTGTA
4081 GCCGTAGTTA GGCCACCACT TCAAGAACTC TGTAAGCACC CTTACATACC TCGCTCTGCT
4141 AATCTGTGTA CCAGTGGCTG CTGCCAGTGG CGATAAGTCG TGTCTTACCG GGTGGAATC
4201 AAGACGATAG TTACCGGATA AGGCGCAGCG GTCGGGCTGA ACGGGGGGTT CGTGACACA
4261 GCCCAGCTTG GAGCGAACGA CCTACACCGA ACTGAGATAC CTACAGCGTG AGCTATGAGA
4321 AAGCGCCACG CTTCGCCAAG GGAGAAAGGC GGACAGGTAT CCGTAAGCG GCAGGGTCGG
4381 AACAGGAGAG CGCACGAGGG AGCTTCCAGG GGGAAACGCC TGGTATCTTT ATAGTCCTGT
4441 CGGGTTTCGC CACCTCTGAC TTGAGCGTCG ATTTTGTGA TGCTCGTCAG GGGGGCGGAG
4501 CCTATGGAAA AACGCCAGCA ACGCGGCCTT TTTACGGTTC CTGGCCTTTT GCTGGCCTTT
4561 TGCTCACATG TTCTTTCCTG CGTTATCCCC TGATTCTGTG GATAACCGTA TTACCGCTTT
4621 TGAGTGAGCT GATACCGCTC GCCGCAGCCG AACGACCGAG CGCAGCGAGT CAGTGAGCGA
4681 GGAAGCGGAA GAGCGCCTGA TGCGGTATTT TCTCCTTACG CATCTGTGCG GTATTTTACA
4741 CCGCATATAT GGTGCACTCT CAGTACAATC TGCTCTGATG CCGCATAGTT AAGCCAGTAT
4801 ACACTCCGCT ATCGCTACGT GACTGGGTCA TGGCTGCGCC CCGACACCCG CCAACACCCG
4861 CTGACGCGCC CTGACGGGCT TGTCTGTCTC CGGCATCCGC TTACAGACAA GCTGTGACCG
4921 TTTCCGGGAG CTGCATGTGT CAGAGGTTTT CACCGTCATC ACCGAAACGC GCGAGGCAGC
4981 TGCGGTAAAG CTCATCAGCG TGGTCGTGAA GCGATTACA GATGTCTGCC TGTTCATCCG
5041 CGTCCAGCTC GTTGAGTTTC TCCAGAAGCG TTAATGTCTG GCTTCTGATA AAGCGGCCCA
5101 TGTTAAGGGC GGTTTTTTCC TGTTTGGTCA CTGATGCCTC CGTGTAAAGG GGATTTCTGT
5161 TCATGGGGGT AATGATACCG ATGAAACGAG AGAGGATGCT CACGATACGG GTTACTGATG
5221 ATGAACATGC CCGGTTACTG GAACGTTGTG AGGGTAAACA ACTGGCGGTA TGGATGCGGC
5281 GGGACCAGAG AAAAATCACT CAGGGTCAAT GCCAGCGCTT CGTTAATACA GATGTAGGTG
5341 TTCCACAGGG TAGCCAGCAG CATCCTGCGA TGCAGATCCG GAACATAATG GTGCAGGGCG
5401 CTGACTTCCG CGTTTCCAGA CTTTACGAAA CACGGAAACC GAAGACCATT CATGTTGTTG
5461 CTCAGGTCGC AGACGTTTTG CAGCAGCAGT CGCTTACGT TCGCTCGCGT ATCGGTGATT
5521 CATTTCTGCTA ACCAGTAAGG CAACCCCGCC AGCCTAGCCG GGTCTCTCAAC GACAGGAGCA
5581 CGATCATGCG CACCCGTGGC CAGGACCCAA CGCTGCCCGA GATGCGCCGC GTGCGGCTGC
5641 TGGAGATGGC GGACGCGATG GATATGTTCT GCCAAGGGTT GGTTTGCGCA TTCACAGTTC
5701 TCCGAAGAA TTGATTGGCT CCAATTCTTG GAGTGGTGAA TCCGTTAGCG AGGTGCGGCC
5761 GGCTTCCATT CAGGTCGAGG TGGCCCGGCT CCATGCACCG CGACGCAACG CCGGGAGGCA
5821 GACAAGGTAT AGGGCGGCGC CTACAATCCA TGCCAACCCG TTCCATGTGC TCGCCGAGGC
5881 GGCATAAATC GCCGTGACGA TCAGCGGTGC AGTGATCGAA GTTAGGCTGG TAAGAGCCGC
5941 GAGCGATCCT TGAAGCTGTC CCTGATGGTC GTCATCTACC TGCCTGGACA GCATGGCCTG
6001 CAACGCGGGC ATCCCGATGC CGCCGGAAGC GAGAAGAATC ATAATGGGGA AGGCCATCCA
6061 GCCTCGCGTC GCGAACGCCA GCAAGACGTA GCCCAGCGCG TCGGCCGCCA TGCCGGCGAT
6121 AATGGCCTGC TTCTCGCCGA AACGTTTGGT GCGGGGACCA GTGACGAAGG CTTGAGCGAG
6181 GCGGTGCAAG ATTCCGAATA CCGCAAGCGA CAGGCCGATC ATCGTCGCGC TCCAGCGAAA
6241 GCGGTCTCTG CCGAAAATGA CCCAGAGCGC TGCCGGCACC TGTCTACGA GTTGATGAT-

FIGURE 36C

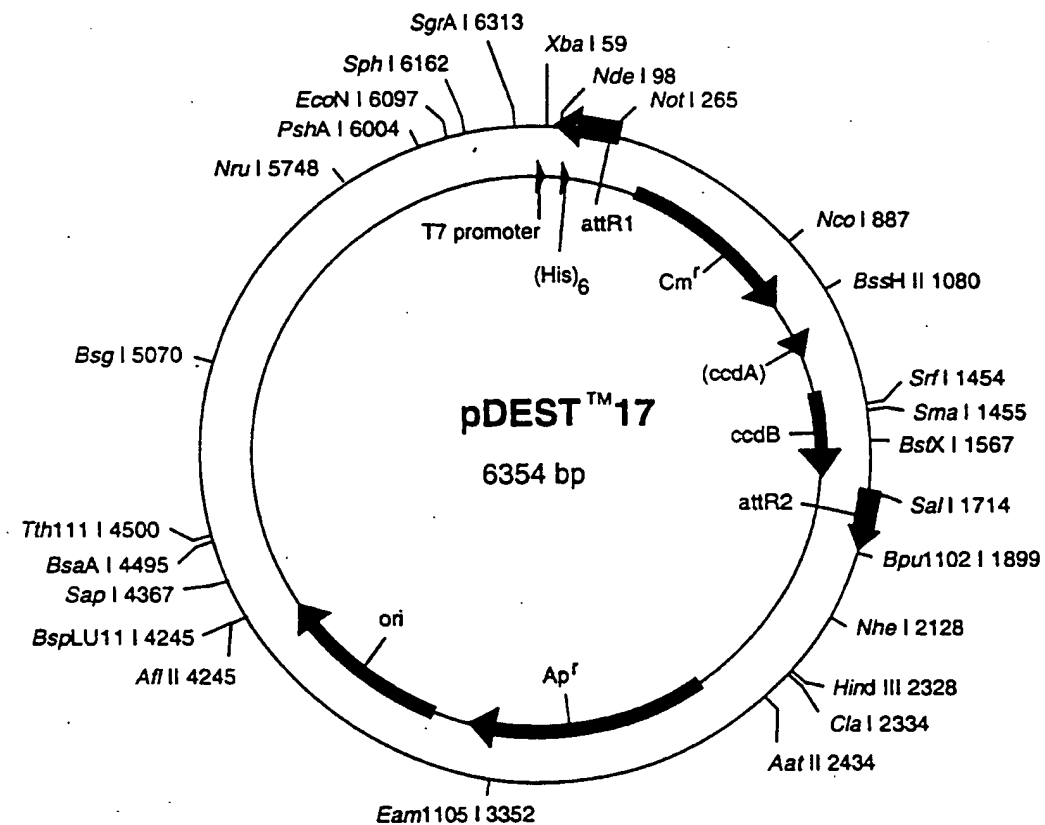
94/240

6301 AAAGAAGACA GTCATAAGTG CGGCGACGAT AGTCATGCCC CGCGCCCACC GGAAGGAGCT
6361 GACTGGGTTG AAGGCTCTCA AGGGCATCGG TCGATCGACG CTCTCCCTTA TGCGACTCCT
6421 GCATTAGGAA GCAGCCCAGT AGTAGGTTGA GGCCGTTGAG CACCGCCGCC GCAAGGAATG
6481 GTGCATGCAA GGAGATGGCG CCCAACAGTC CCCC GGCCAC GGGGCCTGCC ACCATACCCA
6541 CGCCGAAACA AGCGCTCATG AGCCCGAAGT GCGGAGCCCG ATCTTCCCCA TCGGTGATGT
6601 CGGCGATATA GGCGCCAGCA ACCGCACCTG TGGCGCCGGT GATGCCGGCC ACGATGCGTC
6661 CGGCGTAGAG GATCG

FIGURE 36D

95/240

1 gat ccc gcg aaa tta ata cga ctc act ata ggg aga cca caa cgg ttt ccc
 cta ggg cgc ttt aat tat gct gag tga tat ccc tct ggt gtt gcc aaa ggg
 52 tct aga aat aat ttt gtt taa ctt taa gaa gga gat ata cat atg ccg tac
 aga tct tta tta aaa caa att gaa att ctt cct cta tat gta tac agc atg
 Y H H H H H L E S T S L Y K K A
 103 tac cat cac cat cac cat cac ctc gaa tca aca agt ttg tac aaa aaa gct
 atg gta gtg gta gtg gta gtg gag ctt agt tgt tca aac atg ttt ttt cga
 attR1 Int



pDEST17 6354 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
258..134		attR1
367..1026		CmR
1146..1230		inactivated ccdA
1368..1673		ccdB
1714..1838		attR2
2564..3421		ampR

1	CGATCCCGCG	AAATTAATAC	GA	CTCACTAT	AGGGAGACCA	CAACGGTTTC	CCTCTAGAAA
61	TAATTTTGTT	TAACTTTAAG	AAGGAGATAT	ACATATGTCG	TACTACCATC	ACCATCACCA	
121	TCACCTCGAA	TCAACAAGTT	TGTACAAAAA	AGCTGAACGA	GAAACGTAAA	ATGATATAAA	
181	TATCAATATA	TTAAATTAGA	TTTTGCATAA	AAAACAGACT	ACATAATACT	GTAAACACA	
241	ACATATCCAG	TC	ACTATGGC	GGCCGCATTA	GGCACCCAG	GCTTTACACT	TTATGCTTCC
301	GGCTCGTATA	ATGTGTGGAT	TTTGAGTTAG	GATCCGTCGA	GATTTTCAGG	AGCTAAGGAA	
361	GCTAAAATGG	AGAAAAAAT	CACTGGATAT	ACCACCGTTG	ATATATCCCA	ATGGCATCGT	
421	AAAGAACATT	TTGAGGCATT	TCAGTCAGTT	GCTCAATGTA	CCTATAACCA	GACCGTTCAG	
481	CTGGATATTA	CGGCCTTTTT	AAAGACCGTA	AAGAAAAATA	AGCACAAGTT	TTATCCGGCC	
541	TTTATTCACA	TTCTTGCCCG	CCTGATGAAT	GCTCATCCGG	AATTCGGTAT	GGCAATGAAA	
601	GACGGTGAGC	TGGTGATATG	GGATAGTGTT	CACCCTTGTT	ACACCGTTTT	CCATGAGCAA	
661	ACTGAAACGT	TTTCATCGCT	CTGGAGTGAA	TACCACGACG	ATTTCCGGCA	GTTTCTACAC	
721	ATATATTCGC	AAGATGTGGC	GTGTTACGGT	GAAAACCTGG	CCTATTTCCC	TAAAGGGTTT	
781	ATTGAGAATA	TGTTTTTCGT	CTCAGCCAAT	CCCTGGGTGA	GTTTCACCAG	TTTGTATTTA	
841	AACGTGGCCA	ATATGGACAA	CTTCTTCGCC	CCCGTTTTCA	CCATGGGCAA	ATATTATACG	
901	CAAGCGGACA	AGGTGCTGAT	GCCGCTGGCG	ATTGAGGTTT	ATCATGCCGT	CTGTGATGGC	
961	TTCCATGTCT	GCAGAATGCT	TAATGAATTA	CAACAGTACT	GCGATGAGTG	GCAGGGCGGG	
1021	GCGTAAAGAT	CTGGATCCGG	CTTACTAAAA	GCCAGATAAC	AGTATGCGTA	TTTGCGCGCT	
1081	GATTTTTGCG	GTATAAGAAT	ATATACTGAT	ATGTATACCC	GAAGTATGTC	AAAAAGAGGT	
1141	GTGCTATGAA	GCAGCGTATT	ACAGTGACAG	TTGACAGCGA	CAGCTATCAG	TTGCTCAAGG	
1201	CATATATGAT	GTCAATATCT	CCGGTCTGGT	AAGCACAAAC	ATGCAGAATG	AAGCCCGTCG	
1261	TCTGCGTGCC	GAACGCTGGA	AAGCGGAAAA	TCAGGAAGGG	ATGGCTGAGG	TCGCCCCGTT	
1321	TATTGAAATG	AACGGCTCTT	TTGCTGACGA	GAACAGGGAC	TGGTGAAATG	CAGTTTAAGG	
1381	TTTACACCTA	TAAAAGAGAG	AGCCGTTATC	GTCTGTTTGT	GGATGTACAG	AGTGATATTA	
1441	TTGACACGCC	CGGGCGACGG	ATGGTGATCC	CCCTGGCCAG	TGCACGCTCT	CTGTCAGATA	
1501	AAGTCTCCCC	TGAACTTTAC	CCGGTGGTGC	ATATCGGGGA	TGAAAGCTGG	CGCATGATGA	
1561	CCACCGATAT	GGCCAGTGTG	CCGGTCTCCG	TTATCGGGGA	AGAAGTGGCT	GATCTCAGCC	
1621	ACCGCGAAAA	TGACATCAAA	AACGCCATTA	ACCTGATGTT	CTGGGGAATA	TAAATGTCAG	
1681	GCTCCCTTAT	ACACAGCCAG	TCTGCAGGTC	GACCATAGTG	ACTGGATATG	TTGTGTTTTA	
1741	CAGTATTATG	TAGTCTGTTT	TTTATGCAAA	ATCTAATTTA	ATATATTGAT	ATTTATATCA	
1801	TTTTACGTTT	CTCGTTCAGC	TTTCTTGATC	AAAGTGGTTG	ATTCGAGGCT	GCTAACAAAG	
1861	CCCGAAAGGA	AGCTGAGTTG	GCTGCTGCCA	CCGCTGAGCA	ATAACTAGCA	TAACCCCTTG	
1921	GGGCCTCTAA	ACGGGTCTTG	AGGGGTTTTT	TGCTGAAAGG	AGGAACTATA	TCCGGATATC	
1981	CACAGGACGG	GTGTGGTCCG	CATGATCGCG	TAGTCGATAG	TGGCTCCAAG	TAGCGAAGCG	
2041	AGCAGGACTG	GGCGGCGGCC	AAAGCGGTCTG	GACAGTGCTC	CGAGAACGGG	TGCGCATAGA	
2101	AATTGCATCA	ACGCATATAG	CGTAGCAGC	ACGCCATAGT	GACTGGCGAT	GCTGTCCGAA	
2161	TGGACGATAT	CCCGCAAGAG	GCCCGGCAGT	ACCGGCATAA	CCAAGCCTAT	GCCTACAGCA	
2221	TCCAGGGTGA	CGGTGCCGAG	GATGACGATG	AGCGCATTGT	TAGATTTTAT	ACACGGTGCC	
2281	TGACTGCGTT	AGCAATTTAA	CTGTGATAAA	CTACCGCATT	AAAGCTTATC	GATGATAAGC	
2341	TGTCAAACAT	GAGAATTCTT	GAAGACGAAA	GGGCCTCGTG	ATACGCCCTAT	TTTTATAGGT	
2401	TAATGTCATG	ATAATAATGG	TTTCTTAGAC	GTCAGGTGGC	ACTTTTCGGG	GAAATGTGCG	
2461	CGGAACCCCT	ATTTGTTTAT	TTTTCTAAAT	ACATTCAAAT	ATGTATCCGC	TCATGAGACA	
2521	ATAACCCCTG	TAAATGCTTC	AATAATATTG	AAAAAGGAAG	AGTATGAGTA	TTCAACATTT	
2581	CCGTGTCGCC	CTTATCCCTT	TTTTTGCGGC	ATTTTGCTTT	CCTGTTTTTG	CTCACCAGCA	
2641	AACGCTGGTG	AAAGTAAAAG	ATGCTGAAGA	TCAGTTGGGT	GCACGAGTGG	GTTACATCGA-	

FIGURE 37B

97/250

2701 ACTGGATCTC AACAGCGGTA AGATCCTTGA GAGTTTTCGC CCCGAAGAAC GTTTTCCAAT
2761 GATGAGCACT TTAAAGTTC TGCTATGTGG CGCGGTATTA TCCCGTGTG ACGCCGGGCA
2821 AGAGCAACTC GGTCGCCGCA TACACTATTC TCAGAATGAC TTGGTTGAGT ACTCACCAGT
2881 CACAGAAAAG CATCTTACGG ATGGCATGAC AGTAAGAGAA TTATGCAGTG CTGCCATAAC
2941 CATGAGTGAT AACACTGCGG CCAACTTACT TCTGACAACG ATCGGAGGAC CGAAGGAGCT
3001 AACCGCTTTT TTGCACAACA TGGGGGATCA TGTAACTCGC CTGTATCGTT GGGAAACCGA
3061 GCTGAATGAA GCCATACCAA ACGACGAGCG TGACACCACG ATGCCTGCAG CAATGGCAAC
3121 AACGTTGCGC AAATAATTAA CTGGCGAACT ACTTACTCTA GCTTCCCGGC AACAAATTAAT
3181 AGACTGGATG GAGGCGGATA AAGTTGCAGG ACCACTTCTG CGCTCGGCCC TTCCGGCTGG
3241 CTGGTTTATT GCTGATAAAT CTGGAGCCGG TGAGCGTGGG TCTCGCGGTA TCATTGCAGC
3301 ACTGGGGCCA GATGGTAAGC CCTCCCGTAT CGTAGTTATC TACACGACGG GGAGTCAGGC
3361 AACTATGGAT GAACGAAATA GACAGATCGC TGAGATAGGT GCCTCACTGA TTAAGCATTG
3421 GTAACGTGCA GACCAAGTTT ACTCATATAT ACTTTAGATT GATTTAAAC TTCATTTTTA
3481 ATTTAAAAGG ATCTAGGTGA AGATCCTTTT TGATAATCTC ATGACCAAAA TCCCTTAACG
3541 TGAGTTTTTCG TTCCACTGAG CGTCAGACCC CGTAGAAAAG ATCAAAGGAT CTTCTTGAGA
3601 TCCTTTTTTTT CTGCGCGTAA TCTGCTGCTT GCAAACAAAA AAACCACCGC TACCAGCGGT
3661 GGTTTGTTTTG CCGGATCAAG AGCTACCAAC TCTTTTTCCG AAGGTAAC TGCTTCAGCAG
3721 AGCGCAGATA CCAAATACTG TCCTTCTAGT GTAGCCGTAG TTAGGCCACC ACTTCAAGAA
3781 CTCTGTAGCA CCGCCTACAT ACCTCGCTCT GCTAATCCTG TTACCACTGG CTGCTGCCAG
3841 TGGCGATAAG TCGTGTCTTA CCGGGTTGGA CTCAAGACGA TAGTTACCGG ATAAGGCGCA
3901 CGGTCGCGG TGAACGGGGG GTTCGTGCAC ACAGCCAGC TTGGAGCGAA CGACCTACAC
3961 CGAACTGAGA TACCTACAGC GTGAGCTATG AGAAAGCGCC ACGCTTCCCG AAGGGAGAAA
4021 GCGCGACAGG TATCCGGTAA GCGGCAGGGT CGGAACAGGA GAGCGCACGA GGGAGCTTCC
4081 AGGGGGAAAC GCCTGGTATC TTTATAGTCC TGTCGGGTTT CGCCACCTCT GACTTGAGCG
4141 TCGATTTTTG TGATGCTCGT CAGGGGGGCG GAGCCTATGG AAAAACGCCA GCAACGCGGC
4201 CTTTTTACGG TTCCTGGCCT TTTGCTGGCC TTTGCTCAC ATGTTCTTTC CTGCGTTATC
4261 CCCTGATTCT GTGGATAACC GTATTACCGC CTTTGAGTGA GCTGATACCG CTCGCCGACG
4321 CCGAACGACC GAGCGCAGCG AGTCAGTGAG CGAGGAAGCG GAAGAGCGCC TGATGCGGTA
4381 TTTTCTCCTT ACGCATCTGT GCGGTATTTT ACACCGCATA TATGGTGCAC TCTCAGTACA
4441 ATCTGCTCTG ATGCCGCATA GTTAAGCCAG TATACACTCC GCTATCGCTA GGGAGCTGGG
4501 TCATGGCTGC GCCCCGACAC CCGCCAACAC CCGCTGACGC GCCCTGACGG GCTTGTCTGC
4561 TCCCGGCATC CGCTTACAGA CAAGCTGTGA CCGTCTCCGG GAGCTGCATG TGTCAGAGGT
4621 TTTCACCGTC ATCACCAGAA CGCGCAGGCG AGCTGCGGTA AAGCTCATCA GCGTGGTTCG
4681 GAAGCGATTG ACAGATGTCT GCCTGTTTAT CCGCTCCAG CTCGTTGAGT TTCTCCAGAA
4741 GCGTTAATGT CTGGCTTCTG ATAAAGCGGG CCATGTTAAG GCGGGTTTTT TCCTGTTTTG
4801 TCACTGATGC CTCCGTGTAA GGGGGATTTC TGTTTCATGGG GGTAAATGATA CCGATGAAAC
4861 GAGAGAGGAT GCTCACGATA CCGGTTACTG ATGATGAACA TGCCCGGTTA CTGGAACGTT
4921 GTGAGGGTAA ACAACTGGCG GTATGGATGC GGCGGGACCA GAGAAAAATC ACTCAGGTC
4981 AATGCCAGCG CTTGTTAAT ACAGATGTAG GTGTTCCACA GGGTAGCCAG CAGCATCCTG
5041 CGATGCAGAT CCGGAACATA ATGGTGCAGG GCGCTGACTT CCGCGTTTTCC AGACTTTACG
5101 AAACACGGAA ACCGAAGACC ATTCATGTTG TTGCTCAGGT CGCAGACGTT TTGCAGCAGC
5161 AGTCGCTTCA CGTTTCGCTG CGTATCGGTG ATTCATTCTG CTAACCAGTA AGGCAACCCC
5221 GCCAGCCTAG CCGGGTCTC AACGACAGGA GCACGATCAT GCGCACCCGT GGCCAGGACC
5281 CAACGCTGCC CGAGATGCGC CGCGTGCGGC TGCTGGAGAT GGCGGACGCG ATGGATATGT
5341 TCTGCCAAGG GTTGGTTTGC GCATTCACAG TTCTCCGCAA GAATTGATTG GCTCCAATTC
5401 TTGGAGTGGT GAATCCGTTA GCGAGGTGCC GCCGCTTCC ATTCAGGTCG AGGTGGCCCG
5461 GCTCCATGCA CCGCGACGCA ACGCGGGGAG GCAGACAAGG TATAGGGCGG CGCCTACAAT
5521 CCATGCCAAC CCGTTCCATG TGCTCGCCGA GGCGGCATAA ATCGCCGTGA CGATCAGCGG
5581 TCCAGTGATC GAAGTTAGGC TGGTAAGAGC CGCGAGCGAT CCTTGAAGCT GTCCCTGATG
5641 GTCGTCATCT ACCTGCCTGG ACAGCATGGC CTGCAACGCG GGCATCCCGA TGCCGCCGGA
5701 AGCGAGAAGA ATCATAATGG GGAAGGCCAT CCAGCCTCGC GTCGCGAACG CCAGCAAGAC
5761 GTAGCCCAGC GCGTCGGCCG CCATGCCGGC GATAATGGCC TGCTTCTCGC CGAAACGTTT
5821 GGTGGCGGGA CCAGTGACGA AGGCTTGAGC GAGGGCGTGC AAGATTCCGA ATACCGCAAG
5881 CGACAGGCCG ATCATCGTCG CGCTCCAGCG AAAGCGGTCC TCGCCGAAAA TGACCCAGAG
5941 CGCTGCCGGC ACCTGTCCTA CGAGTTGCAT GATAAAGAAG ACAGTCATAA GTGCGGCGAC
6001 GATAGTCATG CCCC GCGCCC ACCGGAAGGA GCTGACTGGG TTGAAGGCTC TCAAGGGCAT
6061 CGGTGATCGC ACGCTCTCCC TTATGCGACT CCTGCATTAG GAAGCAGCCC AGTAGTAGGT
6121 TGAGGCCGTT GAGCACCGCC GCCGCAAGGA ATGGTGCATG CAAGGAGATG GCGCCCAACA-

Figure 37C

98/260

6181 GTCCCCCGGC CACGGGGCCT GCCACCATAC CCACGCCGAA ACAAGCGCTC ATGAGCCCCGA
6241 AGTGGCGAGC CCGATCTTCC CCATCGGTGA TGTCGGCGAT ATAGGCGCCA GCAACCGCAC
6301 CTGTGGCGCC GGTGATGCCG GCCACGATGC GTCCGGCGTA GAGGATCGAG ATCT

FIGURE 37D

Figure 38A: pDEST18

FastBac Transfer Vector with p10
Baculovirus Promoter

1 gaagacctcg gccgtcgccg cgcttgccgg tgggtgctgac cccggatgaa gtggttcgca
cttctggagc cggcagcgcc gcgaacggcc accacgactg gggcctactt caccaagcgt

61 tectcggttt tctggaagge gagcatcggt tggtcgccc ggactctagc tatagttcta
aggagccaaa agaccttcg ctcgtagcaa acaagcgggt cctgagatcg atatcaagat

121 gtggttggtt acgtatcgag caagaaatg aaacgccaaa cgcgtggag cttgtgtgc
caccaaccga tgcatagtc gttctttat tttcggttt gcgaacctc agaacaqacg

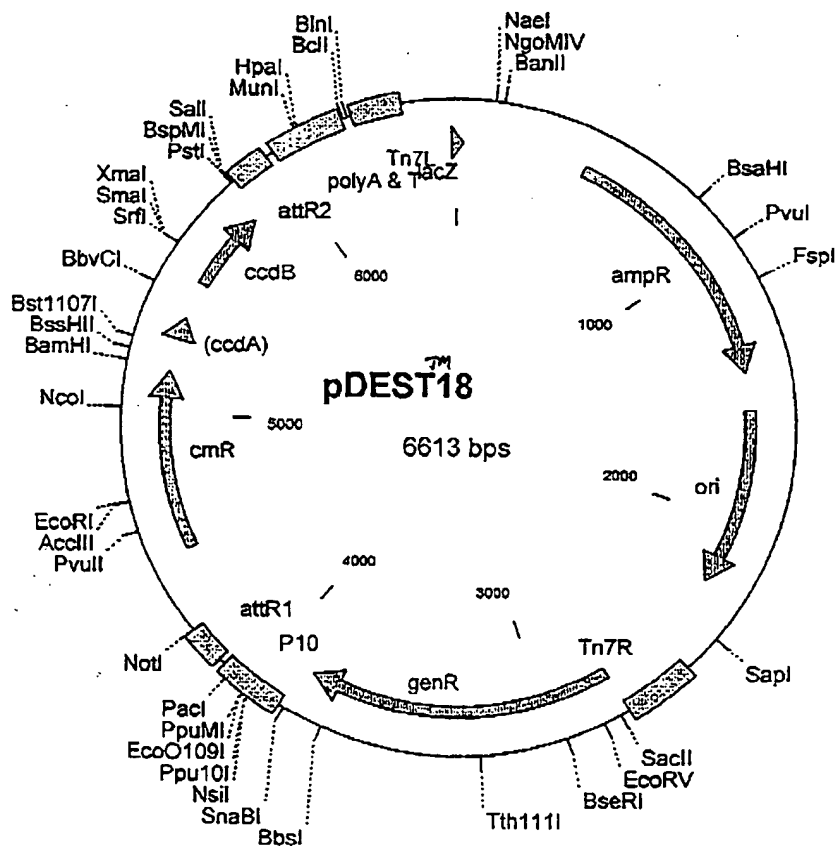
181 ttatgttaca agatccaga aatagcacc attacaaca agggggacta tgaatctatg
ataaaaatgt ttctaagtct ttatgcctag tgaatgtgt tccctctgat acttaatac

241 catcttgagg atgcccggac ctttaattca acccaacaca atatattata gtaaaataga mRNA
glaaaactcc tacggccctg gaaattaagt tgggtgtgt tatataatat caatttatc

301 aattatctat caaatcattt gtataatta taaaatacta tactgtaaat tacatrttat
taataaata gtttagtaaa oataataatta attttatgat atgacattta atgtaaaata

361 ttacaatgag gatcatcaca agtttgtaca aaaaagctga acgagaaacg taaaatgata
aatgttactc ctagtatgt tcaaacatgt ttttcgact tgctctttgc attttactat

Int. ↓ attR1



pDEST18 6613 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
474..1449		ampR
1590..2244		ori
2738..3850		genR
4251..4127		attR1
4501..5160		CmR
5280..5364		inactivated ccdA
5502..5807		ccdB
5848..5972		attR2
6595..25		lacZ

1	GACGCGCCCT	GTAGCGGCGC	ATTAAGCGCG	GCGGGTGTGG	TGGTTACGCG	CAGCGTGACC
61	GCTACACTTG	CCAGCGCCCT	AGCGCCCGCT	CCTTTCGCTT	TCTTCCCTTC	CTTTCGCGC
121	ACGTTTCGCCG	GCTTTCCTCCG	TCAAGCTCTA	AATCGGGGGC	TCCCTTTAGG	GTTCCGATTT
181	AGTGCTTTAC	GGCACCTCGA	CCCCAAAAAA	CTTGATTAGG	GTGATGGTTC	ACGTAGTGGG
241	CCATCGCCCT	GATAGACGGT	TTTTCGCCCT	TTGACGTTGG	AGTCCACGTT	CTTTAATAGT
301	GGACTCTTGT	TCCAACTGG	AACAACACTC	AACCCTATCT	CGGTCTATTC	TTTTGATTTA
361	TAAGGGATT	TGCCGATTTC	GGCCTATTGG	TTAAAAATG	AGCTGATTTA	ACAAAAATTT
421	AACGCGAATT	TTAACAAAAT	ATTAACGTTT	ACAATTTTCAG	GTGGCACTTT	TCGGGGAAAT
481	GTGCGCGGAA	CCCCTATTTC	TTTATTTTTT	TAAATACATT	CAAATATGTA	TCCGCTCATG
541	AGACAATAAC	CCTGATAAAT	GCTTCAATAA	TATTGAAAAA	GGAAGAGTAT	GAGTATTCAA
601	CATTTCCGTG	TCGCCCTTAT	TCCCTTTTTT	GCGGCATTTT	GCCTTCCTGT	TTTTGCTCAC
661	CCAGAAACGC	TGGTGAAAGT	AAAAGATGCT	GAAGATCAGT	TGGGTGCACG	AGTGGGTAC
721	ATCGAACTGG	ATCTCAACAG	CGGTAAGATC	CTTGAGAGTT	TCGCCCCGA	AGAAGTTTT
781	CCAATGATGA	GCACTTTTAA	AGTTCTGCTA	TGTGGCGCGG	TATTATCCCG	TATTGACGCC
841	GGGCAAGAGC	AACTCGGTGC	CCGCATACAC	TATTCTCAGA	ATGACTTGGT	TAGTACTCA
901	CCAGTGCAG	AAAAGCATCT	TACGGATGGC	ATGACAGTAA	GAGAATTATG	CAGTGCTGCC
961	ATAACCATGA	GTGATAACAC	TGCGGCCAAC	TTACTTCTGA	CAACGATCGG	AGGACCGAAG
1021	GAGCTAACCG	CTTTTTTTGCA	CAACATGGGG	GATCATGTAA	CTCGCCTTGA	TCGTTGGGAA
1081	CCGGAGCTGA	ATGAAGCCAT	ACCAAACGAC	GAGCGTGACA	CCACGATGCC	TGTAGCAATG
1141	GCAACAACGT	TGCGCAAAC	ATTAACGTGG	GAACACTTAA	CTCTAGCTTC	CCGGCAACAA
1201	TTAATAGACT	GGATGGAGGC	GGATAAAGTT	GCAGGACCAC	TTCTGCGCTC	GGCCCTTCCG
1261	GCTGGCTGGT	TTATTGCTGA	TAAATCTGGA	GCCGGTGAGC	GTGGGTCTCG	CGGTATCATT
1321	GCAGCACTGG	GGCCAGATGG	TAAGCCCTCC	CGTATCGTAG	TTATCTACAC	GACGGGGAGT
1381	CAGGCAACTA	TGGATGAACG	AAATAGACAG	ATCGCTGAGA	TAGGTGCCTC	ACTGATTAA
1441	CATTGGTAAC	TGTCAGACCA	AGTTTACTCA	TATATACTTT	AGATTGATTT	AAAACITTCAT
1501	TTTTAATTTA	AAAGGATCTA	GGTGAAGATC	CTTTTTGATA	ATCTCATGAC	CAAAATCCCT
1561	TAACGTGAGT	TTTCGTTCCA	CTGAGCGTCA	GACCCCGTAG	AAAAGATCAA	AGGATCTTCT
1621	TGAGATCCTT	TTTTTCTGCG	CGTAATCTGC	TGCTTGCAAA	CAAAAAAACC	ACCGCTACCA
1681	GCGGTGGTTT	GTTTGCCGGA	TCAAGAGCTA	CCAACCTTTT	TTCCGAAGGT	AACTGGCTTC
1741	AGCAGAGCGC	AGATACCAA	TACTGTCCTT	CTAGTGTAGC	CGTAGTTAGG	CCACCACTTC
1801	AAGAACTCTG	TAGCACCGCC	TACATACCTC	GCTCTGCTAA	TCCTGTTACC	AGTGGCTGCT
1861	GCCAGTGCGG	ATAAGTCGTG	TCTTACCGGG	TTGGACTCAA	GACGATAGTT	ACCGGATAAG
1921	GCGCAGCGGT	CGGGCTGAAC	GGGGGGTTCG	TGCACACAGC	CCAGCTTGGA	GCGAACGACC
1981	TACACCGAAC	TGAGATACCT	ACAGCGTGAG	CATTGAGAAA	GCGCCACGCT	TCCCGAAGGG
2041	AGAAAGGCGG	ACAGGTATCC	GGTAAGCGGC	AGGGTCGGAA	CAGGAGAGCG	CACGAGGGAG
2101	CTTCAGGGG	GAAACGCCTG	GTATCTTTAT	AGTCCTGTCTG	GGTTTCGCCA	CCTCTGACTT
2161	GAGCGTCGAT	TTTTGTGATG	CTCGTCAGGG	GGGCGGAGCC	TATGGAAAAA	CGCCAGCAAC
2221	GCGGCTTTT	TACGGTTCCT	GGCCTTTTGC	TGGCCTTTTG	CTCACATGTT	CTTTCCTGCG
2281	TTATCCCTTG	ATTCTGTGGA	TAACCGTATT	ACCGCCTTTG	AGTGAGTGA	TACCGCTCGC
2341	CGCAGCCGAA	CGACCGAGCG	CAGCGAGTCA	GTGAGCGAGG	AAGCGGAAGA	GCGCCTGATG
2401	CGGTATTTTC	TCCTTACGCA	TCTGTGCGGT	ATTTACACACC	GCAGACCAGC	CGCGTAACCT
2461	GGCAAAATCG	GTTACGGTTG	AGTAATAAAT	GGATGCCCTG	CGTAAGCGGG	TGTGGGCGGA-

FIGURE 38B

101/240

2521 CAATAAAGTC TTAAACTGAA CAAAATAGAT CTAAACTATG ACAATAAAGT CTTAAACTAG
2581 ACAGAAATAGT TGTAAGCTGA AATCAGTCCA GTTATGCTGT GAAAAAGCAT ACTGGAATTT
2641 TGTTATGGCT AAAGCAAACCT CTTCAATTTTC TGAAGTGCAA ATTGCCCGTC GTATTAAAGA
2701 GGGGCGTGCC CAAGGGCATG GTAAAGACTA TATTCGCGGC GTTGTGACAA TTTACCSAAC
2761 AACTCCGCGG CCGGGAAGCC GATCTCGGCT TGAACGAATT GTTAGGTGGC GGTACTGGG
2821 TCGATATCAA AGTGCATCAC TTCTTCCCGT ATGCCCAACT TTGTATAGAG AGCCACTGCG
2881 GGATCGTCAC CGTAATCTGC TTGCACGTAG ATCACATAAG CACCAAGCGC GTTGGCCTCA
2941 TGCTTGAGGA GATTGATGAG CGCGGTGGCA ATGCCCTGCC TCCGGTGCTC GCCGGASACT
3001 GCGAGATCAT AGATATAGAT CTCACTACGC GGCTGCTCAA ACCTGGGCAG AACGTAAGCC
3061 GCGAGAGCGC CAACAACCGC TTCTTGGTCG AAGGCAGCAA GCGCGATGAA TGTCTTACTA
3121 CGGAGCAAGT TCCCGAGGTA ATCGGAGTCC GGCTGATGTT GGGAGTAGGT GGCTACGTCT
3181 CCGAACTCAC GACCGAAAAG ATCAAGAGCA GCCCGCATGG ATTTGACTTG GTCAGGSCCG
3241 AGCCTACATG TGCGAATGAT GCCCATACTT GAGCCACCTA ACTTTGTTTT AGGGCGACTG
3301 CCCTGTGCG TAACATCGTT GCTGCTGCGT AACATCGTTG CTGCTCCATA ACATCAACA
3361 TCGACCCACG GCGTAACGCG CTTGCTGCTT GGATGCCCGA GGCATAGACT GTACAAAAA
3421 ACAGTCATAA CAAGCCATGA AAACCGCCAC TCGCGCGTTA CCACCGCTGC GTTCGGTCAA
3481 GGTTCTGGAC CAGTTGCGTG AGCGCATACG CTACTTGCTT TACAGTTTAC GAACCGTACA
3541 GGCTTATGTC AACTGGGTTC GTGCCTTCAT CCGTTTCCAC GGTGTGCGTC ACCCGGCAAC
3601 CTTGGGCAGC AGCGAAGTCG AGGCATTTCT GTCTTGCTG GCGAACGAGC GCAAGGTTTC
3661 GGTCTCCACG CATCGTCAGG CATTGGCGGC CTTGCTGTTT TTCTACGGCA AGGTGCTGTG
3721 CACGGATCTG CCCTGGCTTC AGGAGATCGG AAGACCTCGG CCGTCGCGGC GCTTGCCTGT
3781 GGTGCTGACC CCGGATGAAG TGGTTCGCAT CCTCGGTTTT CTGGAAGGCG AGCATCGTTT
3841 GTTCGCCCCG GACTCTAGCT ATAGTTCTAG TGGTTGGCTA CGTATCGAGC AAGAAAATAA
3901 AACGCCAAAC GCGTTGGAGT CTTGTGTGCT ATTTTACAA AGATTAGAA ATACGCATCA
3961 CTTACAACAA GGGGGACTAT GAAATTATGC ATTTTGAGGA TGCCGGGACC TTAAATTCAA
4021 CCCAACACAA TATATTATAG TTAAATAAGA ATTTATTTATC AAATCATTTC TATATTAAIT
4081 AAAATACTAT ACTGTAAATT ACATTTTATT TACAATGAGG ATCATCACAA GTTTGTACAA
4141 AAAAGCTGAA CGAGAAACGT AAAATGATAT AAATATCAAT ATATTAAAT AGATTTTGCA
4201 TAAAAAACAG ACTACATAAT ACTGTAAAC ACAACATATC CAGTCACTAT GCGGCGCTGT
4261 AAGTTGGCAG CATCACCCGA CGCACTTTGC GCCGAATAAA TACCTGTGAC GGAAGATCAC
4321 TTCGCAGAAT AAATAAATCC TGGTGTCCCT GTTGATACCG GGAAGCCCTG GGCCACTTTT
4381 TGGCGAAAAT GAGACGTTGA TCGGCACGTA AGAGGTTCCA ACTTTCACCA TAATGAATA
4441 AGATCACTAC CGGGCGTATT TTTTGAGTTA TCGAGATTTT CAGGAGCTAA GGAAGCTAAA
4501 ATGGAGAAAA AAATCACTGG ATATACCACC GTTGATATAT CCCAATGGCA TCGTAAGAA
4561 CATTTTGAGG CATTTTCAGT AGTTGTCAA TGTACCTATA ACCAGACCGT TCAGCTGTAT
4621 ATTACGGCCT TTTTAAAGAC CGTAAAGAAA AATAAGCACA AGTTTATCC GGCCTTTATT
4681 CACATTCTTG CCCGCCTGAT GAATGCTCAT CCGGAATTCC GTATGGCAAT GAAAGACGGT
4741 GAGCTGGTGA TATGGGATAG TGTTTCCCTT TGTTACACCG TTTTCCATGA GCAAACGTAA
4801 ACGTTTTTCAT CGCTCTGGAG TGAATACCAC GACGATTTCC GGCAGTTTCT ACACATATAT
4861 TCGCAAGATG TGGCGTGTGA CCGTGAAAAC CTGGCCTATT TCCCTAAAGG GTTTATTGAG
4921 AATATGTTTT TCGTCTCAGC CAATCCCTGG GTGAGTTTCA CCAGTTTGA TTTAAACGTG
4981 GCCAATATGG ACAACTTCTT CGCCCCCGTT TTCACCATGG GCAAATATTA TACGCAAGGC
5041 GACAAGGTGC TGATGCCGCT GGCGATTGAG GTTCATCATG CCGTCTGTGA TGGCTTCCAT
5101 GTCGGCAGAA TGCTTAATGA ATTACAACAG TACTGCGATG AGTGGCAGG CCGGGCCTAA
5161 ACGCGTGGAT CCGGCTTACT AAAAGCCAGA TAACAGTATG CGTATTTGCG CGCTGATTTT
5221 TGCGGTATAA GAATATATAC TGATATGTAT ACCCGAAGTA TGTCAAAAAG AGGTGTCTTA
5281 TGAAGCAGCG TATTACAGTG ACAGTTGACA GCGACAGCTA TCAGTTGCTC AAGGCATATA
5341 TGATGTCAAT ATCTCCGGTC TGGTAAGCAC AACCATGCAG AATGAAGCCC GTCGCTGCG
5401 TGCCGAACGC TGGAAAGCGG AAAATCAGGA AGGGATGGCT GAGGTGCCCC GGTTTATTGA
5461 AATGAACGGC TCTTTTGCTG ACGAGAACAG GGACTGGTGA AATGCAGTTT AAGGTTTACA
5521 CCTATAAAAG AGAGAGCCGT TATCGTCTGT TTGTGGATGT ACAGAGTGAT ATTATTGACA
5581 CGCCCGGGCG ACGGATGGTG ATCCCCCTGG CCAAGTGCAG TCTGCTGTCA GATAAACTCT
5641 CCCCCTGAAC TTACCCGGTG GTGCATATCG GGGATGAAAG CTGGCCCATG ATGACCACCG
5701 ATATGCCAG TGTGCCGGTC TCCGTTATCG GGAAGAAGT GGCTGATCTC AGCCACCGCG
5761 AAAATGACAT CAAAAACGCC ATTAACCTGA TGTTCTGGGG AATATAAATG TCAGGCTCCC
5821 TTATACACAG CCAGTCTGCA GGTGACCAT AGTGAAGTGA TATGTTGTGT TTTACATAT
5881 TATGTAGTCT GTTTTTTATG CAAAATCTAA TTAAATATAT TGATATTAT ATCATTCTAC
5941 GTTTCTCGTT CAGCTTTCTT GTACAAAGTG GTGATAGCTT GTCGAGAAGT ACTAGAGGAT

FIGURE 38C

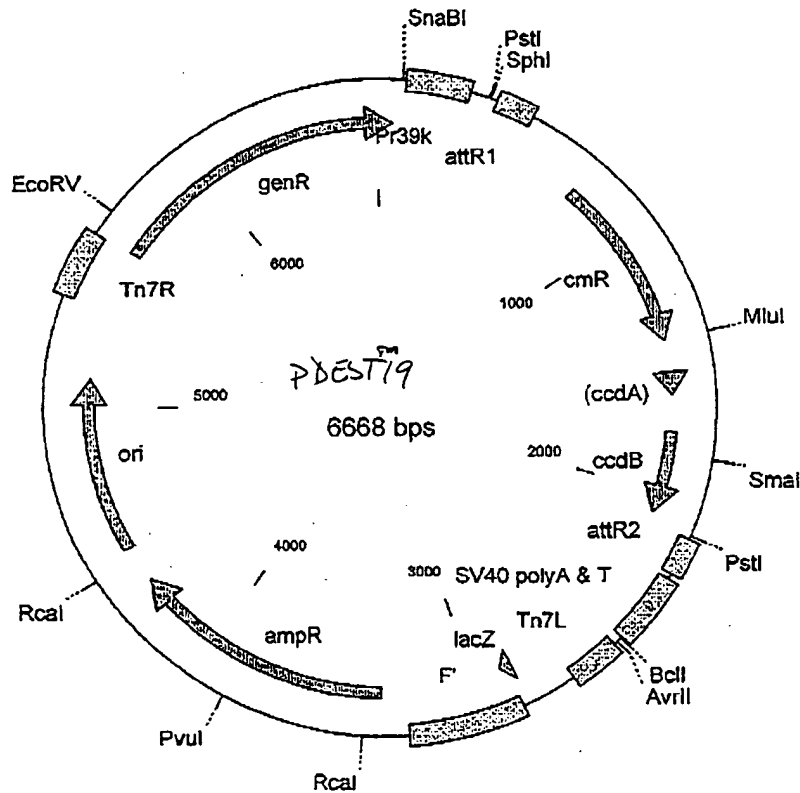
102/240

6001 CATAATCAGC CATAACCACAT TTGTAGAGGT TTTACTTGCT TTAAAAAACC TCCCACACCT
6061 CCCCCTGAAC CTGAAACATA AAATGAATGC AATTGTTGTT GTTAAC TTGT TTATTGCAGC
6121 TTATAATGGT TACAAATAAA GCAATAGCAT CACAAATTC ACAAATAAAG CATTTTTTTC
6181 ACTGCATTCT AGTTGTGGTT TGTCCAAACT CATCAATGTA TCTTATCATG TCTGGATCTG
6241 ATCACTGCTT GAGCCTAGGA GATCCGAACC AGATAAGTGA AATCTAGTTC CAAACTATTT
6301 TGTCATTTTT AATTTTCGTA TTAGCTTACG ACGCTACACC CAGTTCCCAT CTATTTTGTC
6361 ACTCTTCCCT AAATAATCCT TAAAACTCC ATTTCCACCC CTCCCAGTTC CCAACTATTT
6421 TGTCCGCCCA CAGCGGGGCA TTTTCTTCC TGTTATGTTT TTAATCAAAC ATCCTGCCAA
6481 CTCCATGTGA CAAACCGTCA TCTTCGGCTA CTTTTCTCT GTACACAGAAT GAAAATTTTT
6541 CTGTCATCTC TTCGTTATTA ATGTTTGTA TGAAGTGAAT ATCAACGCTT ATTTGCAGCC
6601 TGAATGGCGA ATG

FIGURE 38D

103/240

1 ggtgacgccg tcattcttcc attgtaacgt aaatggcaac ttgtagatga acgcgctgtc
 ccactgcggc agtagaaaagg taacattgca ttaccggttg aacatctact tgcgcgacag
 61 aaaaaaccgg ccagtttctt ccacaaactc gcgcacggct gtctcgtaaa cttttgcgtc
 ttttttgccc ggtcaaagaa ggtgtttgag cgcgtgccga cagagcattt gaaaacgcag
 121 // gcaacaatcg cgatgacctc gtggtatgga aatttttctt aaaaaagtgt cgttcattgtc
 // cgttgtttagc gctactggag caccatacct ttaaaaaaga ttttttcaca gcaagtacag
 181 // ggccggcggcg ttcgcgctcc ggtacgcgcg acgggcacac agcaggacag ctttgtccgg
 // ccgcccgcgc aagcgcgagg ccatgcgcgc tgcccgtgtg tcgtccctgtc ggaacaggcc
 241 ctcgattatc ataaacaatc ctgcaggcat gcaagctgga tcatacaag ttgttacaag
 gagctaatag tatttgtag gacgtccgta cgttcgacct agtagtgttc aaacatgttt
 Int V



104/240

pDEST19 6668 bp (rotated to position 1000)

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
515..391		attR1
765..1424		CmR
1544..1628		inactivated ccdA
1766..2071		ccdB
2112..2236		attR2
2852..2895		lacZ
3344..4319		ampR
4460..5114		ori
5608..52		genR

1	AGTGGTTCGC	ATCCTCGGTT	TTCTGGAAGG	CGAGCATCGT	TTGTTGCCCC	AGGACTCTAG
61	CTATAGTTCT	AGTGGTTGGC	TACGTATATC	AAATACTTGT	AGGTGACGCC	GTCATCTTTC
121	CATTGTAAAC	TAAATGGCAA	CTTGTAGATG	AACGCGCTGT	CAAAAAACCG	GCCAGTTTCT
181	TCCACAAACT	CGCGCACGGC	TGTCTCGTAA	ACTTTTGCGT	CGCAACAATC	GCGATGACCT
241	CGTGGTATGG	AAATTTTTTC	TAAAAAAGTG	TCGTTTCATGT	CGGCGGCGGG	CGCGTTCGCG
301	CTCCGGTACG	CGCGACGGGC	ACACAGCAGG	ACAGCCTTGT	CCGGCTCGAT	TATCATAAAC
361	AATCCTGCAG	GCATGCAAGC	TCGGATCATC	ACAAGTTTGT	ACAAAAAAGC	TGAACGAGAA
421	ACGTAAAATG	ATATAAATAT	CAATATATTA	AATTAGATTT	TGCATAAAAA	ACAGACTACA
481	TAATACTGTA	AAACACAACA	TATCCAGTCA	CTATGGCGGC	CGCTAAGTTG	GCAGCATCAC
541	CCGACGCACT	TTGCGCCGAA	TAAATACCTG	TGACGGAAGA	TCACTTCGCA	GAATAAATAA
601	ATCCTGGTGT	CCCTGTTGAT	ACCGGGAAGC	CCTGGGCCAA	CTTTTGGCGA	AAATGAGACG
661	TTGATCGGCA	CGTAAGAGGT	TCCAACTTTC	ACCATAATGA	AATAAGATCA	CTACCGGGCG
721	TATTTTTTGA	GTTATCGAGA	TTTTCAGGAG	CTAAGGAAGC	TAAAATGGAG	AAAAAATCA
781	CTGGATATAC	CACCGTTGAT	ATATCCCAAT	GGCATCGTAA	AGAACATTTT	GAGGCATTTT
841	AGTCAGTTGC	TCAATGTACC	TATAACCAGA	CCGTTTCAGT	GGATATTACG	GCCTTTTAA
901	AGACCGTAAA	GAAAAATAAG	CACAAGTTTT	ATCCGGCCTT	TATTCACATT	CTTGCCCGCC
961	TGATGAATGC	TCATCCGGAA	TTCCGTATGG	CAATGAAAGA	CGGTGAGCTG	GTGATATGGG
1021	ATAGTGTTCA	CCCTTGTTAC	ACCGTTTTCC	ATGAGCAAAC	TGAAACGTTT	TCATCGCTCT
1081	GGAGTGAATA	CCACGACGAT	TTCCGGCAGT	TTCTACACAT	ATATTCGCAA	GATGTGGCGT
1141	GTTACGGTGA	AAACCTGGCC	TATTTCCCTA	AAGGGTTTAT	TGAGAATATG	TTTTTCGTCT
1201	CAGCCAATCC	CTGGGTGAGT	TTCAACAGTT	TTGATTTTAA	CGTGGCCAAT	ATGGACAACT
1261	TCTTCGCCCC	CGTTTTTCACC	ATGGGCAAAT	ATTATACGCA	AGGCGACAAG	GTGCTGATGC
1321	CGCTGGCGAT	TCAGGTTTAT	CATGCCGTCT	GTGATGGCTT	CCATGTCGGC	AGAATGCTTA
1381	ATGAATTACA	ACAGTACTGC	GATGAGTGGC	AGGCGGGGGC	GTAAACGCGT	GGATCCGGCT
1441	TACTAAAAGC	CAGATAACAG	TATGCGTATT	TGCGCGCTGA	TTTTTGCGGT	ATAAGAATAT
1501	ATACTGATAT	GTATACCCGA	AGTATGTCAA	AAAGAGGTGT	GCTATGAAGC	AGCGTATTAC
1561	AGTGACAGTT	GACAGCGACA	GCTATCAGTT	GCTCAAGGCA	TATATGATGT	CAATATCTCC
1621	GGTCTGGTAA	GCACAACCAT	GCAGAATGAA	GCCCGTCGTC	TGCGTGCCGA	ACGCTGGAAA
1681	GCGGAAAATC	AGGAAGGGAT	GGCTGAGGTC	GCCCGGTTTA	TTGAAATGAA	CGGCTCTTTT
1741	GCTGACGAGA	ACAGGGACTG	GTGAAATGCA	GTTTAAGGTT	TACACCTATA	AAAGAGAGAG
1801	CCGTTATCGT	CTGTTTGTGG	ATGTACAGAG	TGATATTATT	GACACGCCCC	GGCGACGGAT
1861	GGTGATCCCC	CTGGCCAGTG	CACGTCTGCT	GTCAGATAAA	GTCTCCCGTG	AACTTTACCC
1921	GGTGGTGCAT	ATCGGGGATG	AAAGCTGGCG	CATGATGACC	ACCGATATGG	CCAGTGTGCC
1981	GGTCTCCGTT	ATCGGGGAAG	AAGTGGCTGA	TCTCAGCCAC	CGCGAAAATG	ACATCAAAAA
2041	CGCCATTAAC	CTGATGTTCT	GGGGAATATA	AATGTCAGGC	TCCCTTATAC	ACAGCCAGTC
2101	TGCAGGTCTGA	CCATAGTGAC	TGGATATGTT	GTGTTTTACA	GTATTATGTA	GTCTGTTTTT
2161	TATGCAAAAT	CTAATTTAAT	ATATTGATAT	TTATATCATT	TTACGTTTCT	CGTTCAGCTT
2221	TCTTGATACAA	AGTGGTGATC	GAGAAGTACT	AGAGGATCAT	AATCAGCCAT	ACCACATTTG
2281	TAGAGGTTTT	ACTTGCTTTA	AAAAACCTCC	CACACCTCCC	CCTGAACCTG	AAACATAAAA
2341	TGAATGCAAT	TGTTGTTGTT	AACCTGTTTA	TTGCAGCTTA	TAATGGTTAC	AAATAAAGCA
2401	ATAGCATCAC	AAATTTTACA	AATAAAGCAT	TTTTTTCAC	GCATTCTAGT	TGTGGTTTGT
2461	CCAAACTCAT	CAATGTATCT	TATCATGTCT	GGATCTGATC	ACTGCTTGAG	CCTAGGAGAT
2521	CCGAACCAGA	TAAGTGAAAT	CTAGTTCCAA	ACTATTTTGT	CATTTTAAAT	TTTCGTATTA
2581	GCTTACGACG	CTACACCCAG	TTCCCATCTA	TTTTGTCACT	CTTCCCTAAA	TAATCCTTAA

FIGURE 39B

105/240

2641 AAACTCCATT TCCACCCCTC CCAGTTCCCA ACTATTTTGT CCGCCCACAG CGGGGCATTT
2701 TTCTTCTGT TATGTTTTTA ATCAAACATC CTGCCAACTC CATGTGACAA ACCGTCATCT
2761 TCGGCTACTT TTTCTCTGTC ACAGAATGAA AATTTTTCTG TCATCTCTTC GTTATTAATG
2821 TTTGTAATTG ACTGAATATC AACGCTTATT TGCAGCCTGA ATGGCGAATG GACGCGCCCT
2881 GTAGCGGCGC ATTAAGCGCG GCGGGTGTGG TGGTTACGCG CAGCGTGACC GCTACACTTG
2941 CCAGCGCCCT AGCGCCCGCT CCTTTCGCTT TCTTCCCTTC CTTTCTCGCC ACGTTCGCCG
3001 GCITTTCCCG TCAAGCTCTA AATCGGGGGT TCCCTTAGG GTTCCGATTT AGTGCTTTAC
3061 GGCACCTCGA CCCCCAAAAA CTTGATTAGG GTGATGGTTC ACGTAGTGGG CCATCGCCCT
3121 GATAGACGGT TTTTCGCCCT TTGACGTTGG AGTCCACGTT CTTTAATAGT GGACTCTTGT
3181 TCCAAACTGG AACAACTC AACCCTATCT CGGTCTATTC TTTTGATTTA TAAGGGATTT
3241 TGCCGATTTT GGCCTATTGG TTAAAAAATG AGCTGATTTA ACAAATTTT AACGCGAATT
3301 TTAACAAAT ATTAACGTTT ACAATTTTCA GTGGCACTTT TCGGGGAAAT GTGCGCGGAA
3361 CCCCTATTTG TTTATTTTTC TAAATACATT CAAATATGTA TCCGCTCATG AGACAATAAC
3421 CCTGATAAAT GCTTCAATAA TATTGAAAAA GGAAGAGTAT GAGTATTCAA CATTTCGGTG
3481 TCGCCCTTAT TCCCTTTTTT GCGGCATTTT GCCTTCCTGT TTTTGCTCAC CCAGAAACGC
3541 TGGTGAAAGT AAAAGATGCT GAAGATCAGT TGGGTGCACG AGTGGGTAC ATCGAACTGG
3601 ATCTCAACAG CGGTAAGATC CTTGAGAGTT TTCGCCCCGA AGAACGTTTT CCAATGATGA
3661 GCACTTTTAA AGTTCTGCTA TGTGGCGCGG TATTATCCCG TATTGACGCC GGGCAAGAGC
3721 AACTCGGTCT CCGCATACAC TATTCTCAGA ATGACTTGGT TGAGTACTCA CCAGTCACAG
3781 AAAAGCATCT TACGGATGGC ATGACAGTAA GAGAATTATG CAGTGTGCC ATAACCATGA
3841 GTGATAACAC TGCGGCCAAC TTACTTCTGA CAACGATCGG AGGACCGAAG GAGCTAACCG
3901 CTTTTTTGCA CAACATGGGG GATCATGTAA CTCGCCCTGA TCGTTGGGAA CCGGAGCTGA
3961 ATGAAGCCAT ACCAAACGAC GAGCGTGACA CCACGATGCC TGTAGCAATG GCAACACGT
4021 TTGCGCAAACT ATTAACGTTT GAACTACTTA CTCTAGCTTC CCGGCAACAA TTAATAGACT
4081 GGATGGAGGC GGATAAAGTT GCAGGACCAC TTCTGCGCTC GGCCCTTCCG GCTGGCTGGT
4141 TTATTGCTGA TAAATCTGGA GCCGGTGAGC GTGGGTCTCG CCGTATCATT GCAGCACTGG
4201 GGCCAGATGG TAAGCCCTCC CGTATCGTAG TTATCTACAC GACGGGGAGT CAGGCAACTA
4261 TGGATGAACG AAATAGACAG ATCGCTGAGA TAGGTGCCTC ACTGATTAAG CATTGGTAAC
4321 TGTCAGACCA AGTTTACTCA TATATACTTT AGATTGATTT AAAACTTCAT TTTTAATTTA
4381 AAAGGATCTA GGTGAAGATC CTTTTTGATA ATCTCATGAC CAAAATCCCT TAACGTGAGT
4441 TTTGTTTCCA CTGAGCGTCA GACCCCGTAG AAAAGATCAA AGGATCTTCT TGAGATCCTT
4501 TTTTCTGCG CGTAATCTGC TGCTTGCAA CAAAAAACC ACCGCTACCA GCGGTGGTTT
4561 GTTTGCCGGA TCAAGAGCTA CCAACTCTTT TTCCGAAGGT AACTGGCTTC AGCAGAGCGC
4621 AGATAACAAA TACTGTCTT CTAGTGTAGC CGTAGTTAGG CCACCACTTC AAGAACTCTG
4681 TAGCACCGCC TACATACCTC GCTCTGCTAA TCCTGTTACC AGTGGCTGCT GCCAGTGGCG
4741 ATAAGTCGTG TCTTACCGGG TTGGACTCAA GACGATAGTT ACCGGATAAG GCGCAGCGGT
4801 CGGGCTGAAC GGGGGGTTTC TGCACACAGC CCAGCTTGA GCGAACGACC TACACCGAAC
4861 TGAGATACCT ACAGCGTGAG CATTGAGAAA GCGCCACGCT TCCCGAAGGG AGAAAGCGCG
4921 ACAGGTATCC GGTAAGCGGC AGGGTCGGAA CAGGAGAGCG CACGAGGGAG CTTCCAGGGG
4981 GAAACGCCCTG GTATCTTTAT AGTCTGTGCG GGTTCGCCA CCTCTGACTT GAGCGTCGAT
5041 TTTTGTGATG CTCGTCAGGG GGGCGGAGCC TATGGAAAAA CGCCAGCAAC GCGGCCTTTT
5101 TACGGTTCCT GGCCTTTTGC TGGCCTTTTG CTCACATGTT CTTTCCTGCG TTATCCCCTG
5161 ATTCTGTGGA TAACCGTATT ACCGCTTTG AGTGAGCTGA TACCGCTCGC CGCAGCCGAA
5221 CGACCGAGCG CAGCGAGTCA GTGAGCGAGG AAGCGGAAGA GCGCCTGATG CCGTATTTTC
5281 TCCTTACGCA TCTGTGCGGT ATTTACACCC GCAGACCAGC GCGTAACCT GGCAAAATCG
5341 GTTACGGTTG AGTAATAAAT GGATGCCCTG CGTAAGCGGG TGTGGGCGGA CAATAAAGTC
5401 TTAAACTGAA CAAAATAGAT CTAACTATG ACAATAAAGT CTTAACTAG ACAGAATAGT
5461 TGTAAGTGA AATCAGTCCA GTTATGCTGT GAAAAAGCAT ACTGGACTTT TGTTATGGCT
5521 AAAGCAAAT CTTCAATTTT TGAAGTGCAA ATTGCCCCTC GTATTAAAGA GGGGCGTGGC
5581 CAAGGGCATG GTAAAGACTA TATTCGCGGC GTTGTGACAA TTTACCGAAC AACTCCGCGG
5641 CCGGGAAGCC GATCTCGGCT TGAACGAATT GTTAGGTGGC GGTACTTGGG TCGATATCAA
5701 AGTGCATCAC TTCTTCCCGT ATGCCCAACT TTGTATAGAG AGCCACTGCG GGATCGTCAC
5761 CGTAATCTGC TTGCACGTAG ATCACATAAG CACCAAGCGC GTTGGCCTCA TGCTTGAGGA
5821 GATTGATGAG CGCGGTGGCA ATGCCCTGCC TCCGGTGCTC GCCGGAGACT GCGAGATCAT
5881 AGATATAGAT CTCACTACGC GGCTGCTCAA ACCTGGGCAG AACGTAAGCC GCGAGAGCGC
5941 CAACAACCGC TTCTTGGTCG AAGGCAGCAA GCGCGATGAA TGTCTTACTA CGGAGCAAGT
6001 TCCCAGAGTA ATCGGAGTCC GGCTGATGTT GGGAGTAGGT GGCTACGTCT CCGAACTCAC
6061 GACCGAAAAG ATCAAGAGCA GCCCGCATGG ATTTGACTTG GTCAGGGCCG AGCCTACATG-

Figure 39C

106/240

6121 TCGGAATGAT GCCCATACTT GAGCCACCTA ACTTTGTTTT AGGGCGACTG CCCTGCTGCG
6181 TAACATCGTT GCTGCTGCGT AACATCGTTG CTGCTCCATA ACATCAAACA TCGACCCACG
6241 GCGTAACGCG CTTGCTGCTT GGATGCCCCG GGCATAGACT GTACAAAAAA ACAGTCATAA
6301 CAAGCCATGA AAACCGCCAC TCGCGCGTTA CCACCGCTGC GTTCGGTCAA GGTTCTGGAC
6361 CAGTTGCGTG AGCGCATACG CTACTTGCAT TACAGTTTAC GAACCGAACA GGCTTATGTC
6421 AACTGGGTTC GTGCCTTCAT CCGTTTCCAC GGTGTGCGTC ACCCGGCAAC CTTGGGCAGC
6481 AGCGAAGTCG AGGCATTCTT GTCTTGCGTG GCGAACGAGC GCAAGGTTTC GGTCTCCACG
6541 CATCGTCAGG CATGGCGGC CTTGCTGTTT TTCTACGGCA AGGTGCTGTG CACGGATCTG
6601 CCCTGGCTTC AGGAGATCGG AAGACCTCGG CCGTCGCGGC GCTTGCCGGT GGTGCTGACC
6661 CCGGATGA

FIGURE 39A

107/240

Figure 40A: pDEST20 Glutathione-S-transferase Fusion with Polyhedron Promoter for Baculovirus Expression

430 ggc tac gta tac tcc gga ata tta ata gat cat gga gat aat taa aat gat
 ccg atg cat atg agg cct tat aat tat cta gta cct cta tta att tta cta

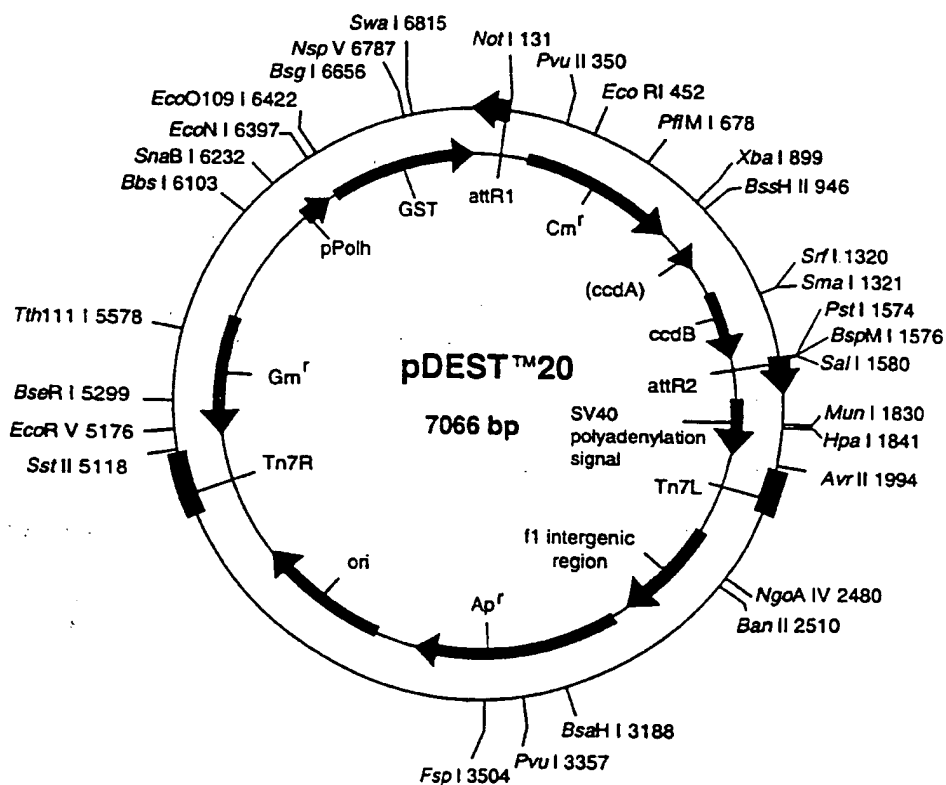
481 aac cat ctc gca aat .aaa taa gta ttt tac tgt ttt cgt aac agt ttt gta
 ttg gta gag cgt tta ttt att cat aaa atg aca aaa gca ttg tca aaa cat

532 ata aaa aaa cct ata aat att ccg gat tat tca tac cgt ccc acc atc ggg
 tat ttt ttt gga tat tta taa ggc cta ata agt atg gca ggg tgg tag ccc

Start Transl. mRNA → A P T - - - GST - -
 583 cgc gga tcc atg gcc cct ata cta ggt tat tgg aaa att aag ggc ctt gtg
 gcg cct agg tac cgg gga tat gat cca ata acc ttt taa ttc ccg gaa cac

1246 S D L V P R H N Q T S L Y K R A
 tcg gat ctg gtt ccg cgt cat aat caa aca agt ttg tac aaa aaa gct gaa
 agc cta gac caa ggc gca gta tta gtt tgt tca aac atg ttt ttt cga ctt

1297 cga gaa acg taa aat gat ata aat atc aat ata tta aat tag at
 gct ctt tgc att tta cta tat tta tag tta tat aat tta atc ta



108/240

pDEST20 7066 bp (rotated to position 5800)

<u>Location (Base Nos.)</u>			<u>Gene Encoded</u>			
592..1263			GST			
1397..1273			attR1			
1506..2165			CmR			
2285..2369			inactivated ccdA			
2507..2812			ccdB			
2853..2977			attR2			
4214..5064			ampR			
5263..5843			ori			
1	CCACTGCGCC	GTTACCACCG	CTGCGTTCGG	TCAAGGTTCT	GGACCAGTTG	CGTGAGCGCA
61	TACGCTACTT	GCATTACAGT	TTACGAACCG	AACAGGCTTA	TGTCAACTGG	GTTCGTGCCT
121	TCATCCGTTT	CCACGGTGTG	CGTCACCCGG	CAACCTTGGG	CAGCAGCGAA	GTCCGAGGCAT
181	TTCTGTCTCG	GCTGGCGAAC	GAGCGCAAGG	TTTCGGTCTC	CACGCATCGT	CAGGCATTGG
241	CGGCCCTTGCT	GTTCTTCTAC	GGCAAGGTGC	TGTGCACGGA	TCTGCCCTGG	CTTCAGGAGA
301	TCGGAAGACC	TCGGCCGTCG	CGGCGCTTGC	CGGTGGTGCT	GACCCCGGAT	GAAGTGGTTC
361	GCATCCTCGG	TTTTCTGGAA	GGCAGCATC	GTTTGTTCGC	CCAGGACTCT	AGCTATAGTT
421	CTAGTGGTTG	GCTACGTATA	CTCCGGAATA	TTAATAGATC	ATGGAGATAA	TTAAAATGAT
481	AACCATCTCG	CAAATAAATA	AGTATTTTAC	TGTTTTCGTA	ACAGTTTTGT	AATAAAAAAA
541	CCTATAAATA	TTCCGGATTA	TTCATACCGT	CCCACCATCG	GGCGCGGATC	CATGGCCCCCT
601	ATACTAGGTT	ATTGGAATAA	TAAGGGCCTT	GTGCAACCCA	CTCGACTTCT	TTTGAATAT
661	CTTGAAGAAA	AATATGAAGA	GCATTTGTAT	GAGCGCGATG	AAGGTGATAA	ATGGCGAAAC
721	AAAAAGTTTG	AATTGGGTTT	GGAGTTTCCC	AATCTTCCTT	ATTATATTGA	TGGTGATGTT
781	AAATTAACAC	AGTCTATGGC	CATCATACGT	TATATAGCTG	ACAAGCACAA	CATGTTGGGT
841	GGTTGTCCAA	AAGAGCGTGC	AGAGATTTCA	ATGCTTGAAG	GAGCGGTTTT	GGATATTAGA
901	TACGGTGTTC	CGAGAATTGC	ATATAGTAAA	GACTTTGAAA	CTCTCAAAGT	TGATTTTCTT
961	AGCAAGCTAC	CTGAAATGCT	GAAAATGTTC	GAAGATCGTT	TATGTCATAA	AACATATTTA
1021	AATGGTGATC	ATGTAACCCA	TCCTGACTTC	ATGTTGTATG	ACGCTCTTGA	TGTTGTTTTA
1081	TACATGGACC	CAATGTGCCT	GGATGCGTTC	CCAAAATTAG	TTTGTTTTAA	AAAACGTATT
1141	GAAGCTATCC	CACAAATTGA	TAAGTACTTG	AAATCCAGCA	AGTATATAGC	ATGGCCTTTG
1201	CAGGGCTGGC	AAGCCACGTT	TGGTGGTGGC	GACCATCCTC	CAAAATCGGA	TCTGGTTCCG
1261	CGTCATAATC	AAACAAGTTT	GTACAAAAAA	GCTGAACGAG	AAACGTAAAA	TGATATAAAT
1321	ATCAATATAT	TAAATTAGAT	TTTGCATAAA	AAACAGACTA	CATAATACTG	TAAAACACAA
1381	CATATCCAGT	CACTATGGCG	GCCGCATTAG	GCACCCAGG	CTTTACACTT	TATGCTTCCG
1441	GCTCGTATGT	TGTGTGGATT	TTGAGTTAGG	ATCCGGCGAG	ATTTTCAGGA	GCTAAGGAAG
1501	CTAAAATGGA	GAAAAAATC	ACTGGATATA	CCACCGTTGA	TATATCCCAA	TGGCATCGTA
1561	AAGAACATTT	TGAGGCATTT	CAGTCAGTTG	CTCAATGTAC	CTATAACCAG	ACCGTTCAGC
1621	TGGATATTAC	GGCCTTTTTA	AAGACCGTAA	AGAAAAATAA	GCACAAGTTT	TATCCGGCCT
1681	TTATTCACAT	TCTTGCCCGC	CTGATGAATG	CTCATCCGGA	ATTCCGTATG	GCAATGAAAG
1741	ACGGTGAGCT	GGTGATATGG	GATAGTGTTC	ACCCTTGTTA	CACCGTTTTT	CATGAGCAAA
1801	CTGAAACGTT	TTCATCGCTC	TGGAGTGAAT	ACCACGACGA	TTTCCGGCAG	TTTCTACACA
1861	TATATTCGCA	AGATGTGGCG	TGTTACGGTG	AAAACCTGGC	CTATTTCCCT	AAAGGGTTTTA
1921	TTGAGAATAT	GTTTTTCGTC	TCAGCCAATC	CCTGGGTGAG	TTTCACCAGT	TTTGATTTAA
1981	ACGTGGCCAA	TATGGACAAC	TTCTTCGCCC	CCGTTTTTAC	CATGGGCAAA	TATTATACGC
2041	AAGGCGACAA	GGTGCTGATG	CCGCTGGCGA	TTCAGGTTCA	TCATGCCGTC	TGTGATGGCT
2101	TCCATGTCCG	CAGAATGCTT	AATGAATTAC	AACAGTACTG	CGATGAGTGG	CAGGGCGGGG
2161	CGTAATCTAG	AGGATCCGGC	TTACTAAAAG	CCAGATAACA	GTATGCGTAT	TTGCGCGCTG
2221	ATTTTTGCGG	TATAAGAATA	TATACTGATA	TGTATAACCG	AAGTATGTCA	AAAAGAGGTG
2281	TGCTATGAAG	CAGCGTATTA	CAGTGACAGT	TGACAGCGAC	AGCTATCAGT	TGCTCAAGGC
2341	ATATATGATG	TCAATATCTC	CGGTCTGGTA	AGCACAACCA	TGCAGAATGA	AGCCCGTCGT
2401	CTGCGTGCCG	AACGCTGGAA	AGCGGAAAAA	CAGGAAGGGA	TGGCTGAGGT	CGCCCGGTTT
2461	ATTGAAATGA	ACGGCTCTTT	TGCTGACGAG	AACAGGGACT	GGTGAATATG	AGTTTAAGGT
2521	TTACACCTAT	AAAAGAGAGA	GCCGTTATCG	TCTGTTTGTG	GATGTACAGA	GTGATATTAT
2581	TGACACGCCC	GGGCGACGGA	TGGTGATCCC	CCTGGCCAGT	GCACGTCTGC	TGTCAGATAA
2641	AGTCTCCCGT	GAACCTTACC	CGGTGGTGCA	TATCGGGGAT	GAAAGCTGGC	GCATGATGAC-

Figure 40B

109/240

2701 CACCGATATG GCCAGTGTGC CGGTCTCCGT TATCGGGGAA GAAGTGGCTG ATCTCAGCCA
2761 CCGCGAAAAT GACATCAAAA ACGCCATTAA CCTGATGTTT TGGGGAATAT AAATGTCAGG
2821 CTCCCTTATA CACAGCCAGT CTGCAAGTGC ACCATAGTGA CTGGATATGT TGTGTTTTAC
2881 AGTATTATGT AGTCTGTTTT TTATGCAAAA TCTAATTTAA TATATTGATA TTTATATCAT
2941 TTTACGTTTC TCGTTCAGCT TTCTTGATACA AAGTGGTTTT ATAGCTTGTC GAGAAGTACT
3001 AGAGGATCAT AATCAGCCAT ACCACATTTG TAGAGGTTTT ACTTGCTTTA AAAAACCTCC
3061 CACACCTCCC CCTGAACCTG AAACATAAAA TGAATGCAAT TGTGTTGTTT AACTTGTTTA
3121 TTGCAGCTTA TAATGGTTAC AAATAAAGCA ATAGCATCAC AAATTTTACA AATAAAGCAT
3181 TTTTTTCACT GCATTCTAGT TGTGGTTTGT CCAAACCTCAT CAATGTATCT TATCATGTCT
3241 GGATCTGATC ACTGCTTGAG CCTAGGAGAT CCGAACCAGA TAAGTGAAAT CTAGTTCCAA
3301 ACTATTTTGT CATTTTTAAT TTTCGTATTA GCTTACGACG CTACACCCAG TTCCCATCTA
3361 TTTTGTCACT CTTCCCTAAA TAATCCTTAA AAACCTCCATT TCCACCCCTC CCAGTTCCCA
3421 ACTATTTTGT CCGCCACAG CGGGGCATTT TTCTTCCTGT TATGTTTTTA ATCAAACATC
3481 CTGCCAACTC CATGTGACAA ACCGTCATCT TCGGCTACTT TTTCTCTGTC ACAGAATGAA
3541 AATTTTTCTG TCATCTCTTC GTTATTAATG TTTGTAATTG ACTGAATATC AACGCTTATT
3601 TGCAGCCTGA ATGGCGAATG GACGCGCCCT GTAGCGGCGC ATTAAGCGCG CGCGGTGTGG
3661 TGGTTACGCG CAGCGTGACC GCTACACTTG CCAGCGCCCT AGCGCCCGCT CCTTTCGCTT
3721 TCTTCCCTTC CTTTCTCGCC ACGTTCGCGG GCTTTCCTCG TCAAGCTCTA AATCGGGGGC
3781 TCCCTTTAGG GTTCCGATTT AGTGCTTTAC GGCACCTCGA CCCCCAAAAA CTGATTAGG
3841 GTGATGGTTC ACGTAGTGGG CCATCGCCCT GATAGACGGT TTTTCGCCCT TTGACGTTGG
3901 AGTCCACGTT CTTTAATAGT GGAATCTTGT TCCAACTGG AACACACTC AACCTATCT
3961 CGGTCTATTC TTTTGATTTA TAAGGGATTT TGCCGATTTT GGCCTATTGG TTAATAAATG
4021 AGCTGATTTA AAAAAAATTT AACCGCAATT TTAACAAAT ATTAACGTTT ACAATTTTAC
4081 GTGGCACTTT TCGGGGAAAT GTGCGCGGAA CCCCTATTG TTTATTTTTT TAAATACATT
4141 CAAATATGTA TCCGCTCATG AGACAATAAC CCTGATAAAT GCTTCAATAA TATTGAAAAA
4201 GGAAGAGTAT GAGTATTCAA CATTTCCGTG TCGCCCTTAT TCCCTTTTTT GCGGCATTTT
4261 GCCTTCCTGT TTTTGCTCAC CCAGAAACGC TGGTGAAAGT AAAAGATGCT GAAGATCAGT
4321 TGGGTGCACG AGTGGGTTAC ATCGAACTGG ATCTCAACAG CGGTAAGATC CTGAGAGTT
4381 TTCGCCCCGA AGAAGCTTTT CCAATGATGA GCACTTTTAA AGTTCTGCTA TGTGGCGCGG
4441 TATTATCCCG TATTGACGCC GGGCAAGAGC AACTCGGTCG CCGCATACAC TATTCTCAGA
4501 ATGACTTGGT TGAGTACTCA CCAGTCACAG AAAAGCATCT TACGGATGGC ATGACAGTAA
4561 GAGAATTATG CAGTGCTGCC ATAACCATGA GTGATAACAC TGCGGCCAAC TTACTTCTGA
4621 CAACGATCGG AGGACCGAAG GAGCTAACCG CTTTTTTTGA CAACATGGGG GATCATGTAA
4681 CTCGCCTTGA TCGTTGGGAA CCGGAGCTGA ATGAAGCCAT ACCAAACGAC GAGCGTGACA
4741 CCACGATGCC TGTAGCAATG GCAACAACGT TCGCCAAAC ATTAACTGGC GAACTACTTA
4801 CTCTAGCTTC CCGGCAACAA TTAATAGACT GGATGGAGGC GGATAAAGTT GCAGGACCAC
4861 TTCTGCGCTC GGCCCTTCCG GCTGGCTGGT TTATTGCTGA TAAATCTGGA GCCGGTGAGC
4921 GTGGGTCTCG CGGTATCATT GCAGCACTGA GGCCAGATGG TAAGCCCTCC CGTATCTGAG
4981 TTATCTACAC GACGGGGAGT CAGGCACTGA TGGATGAACG AAATAGACAG ATCGCTGAGA
5041 TAGGTGCCTC ACTGATTAG CATTTGTAAC TGTCAGACCA AGTTTACTCA TATATACITT
5101 AGATTGATTT AAAACTTCAT TTTTAATTTA AAAGGATCTA GGTGAAGATC CTTTTTGATA
5161 ATCTCATGAC CAAATCCCT TAACGTGAGT TTTGCTTCCA CTGAGCGTCA GACCCCGTAG
5221 AAAAGATCAA AGGATCTTCT TGAGATCCTT TTTTCTGCG CGTAATCTGC TGCTTGCAAA
5281 CAAAAAACC ACCGCTACCA GCGGTGGTTT GTTTGCCGGA TCAAGAGCTA CCAACTCTTT
5341 TTCCGAAGGT AACTGGCTTC AGCAGAGCGC AGATAACAAA TACTGTCTTT CTAGTGAGC
5401 CGTAGTTAGG CCACCCTTC AAGAACTCG TAGCACCGCC TACATACCTC GCTCTGCTAA
5461 TCCTGTTACC AGTGGCTGCT GCCAGTGGCG ATAAGTCGTG TCTTACCGGG TTGGACTCAA
5521 GACGATAGTT ACCGATAAG GCGCAGCGGT CCGGCTGAAC GGGGGGTTTC TGCACACAGC
5581 CCAGCTTGGA GCGAACGACC TACACCGAAC TGAGATACCT ACAGCGTGAG CATTGAGAAA
5641 GCGCCACGCT TCCCGAAGGG AGAAAGGCGG ACAGGTATCC GGTAAGCGGC AGGGTCGGAA
5701 CAGGAGAGCG CACGAGGGAG CTTCCAGGGG GAAACGCTG GTATCTTTAT AGTCCTGTCT
5761 GGTTTCGCCA CCTCTGACTT GAGCGTCGAT TTTTGTGATG CTCGTCAGGG GGGCGGAGCC
5821 TATGGAAAAA CGCCAGCAAC GCGGCTTTT TACGGTTTCT GGCCTTTTTC TGGCCTTTTG
5881 CTCACATGTT CTTTCTGCG TTATCCCTG ATTCTGTGGA TAACCGTATT ACCGCTTTTG
5941 AGTGAGCTGA TACCGCTCGC GCGAGCCGAA CGACCGAGCG CAGCGAGTCA GTGAGCGAGG
6001 AAGCGGAAGA GCGCCTGATG CCGTATTTTC TCCTTACGCA TCTGTGCGGT ATTTACACAC
6061 GCAGACCAGC CGCGTAACCT GGCAAAATCG GTTACGGTTG AGTAATAAAT GGATGCCCTG
6121 CGTAAGCGGG TGTGGGCGGA CAATAAAGTC TTAAACTGAA CAAATAGAT CTAACTATG-

FIGURE 40C

110/240

6181 ACAATAAAGT CTTAAACTAG ACAGAATAGT TGTAAACTGA AATCAGTCCA GTTATGCTGT
6241 GAAAAAGCAT ACTGGACTTT TGTTATGGCT AAAGCAAACCT CTCATTTTC TGAAGTGCAA
6301 ATTGCCCCGTC GTATTAAAGA GGGGCGTGGC CAAGGGCATG GTAAAGACTA TATTCGCGGC
6361 GTTGTGACAA TTTACCGAAC AACTCCGCGG CCGGGAAGCC GATCTCGGCT TGAACGAATT
6421 GTTAGGTGGC GGTACTTGGG TCGATATCAA AGTGCATCAC TTCTTCCCGT ATGCCCAACT
6481 TTGTATAGAG AGCCACTGCG GGATCGTCAC CGTAATCTGC TTGCACGTAG ATCACATAAG
6541 CACCAAGCGC GTTGGCCTCA TGCTTGAGGA GATTGATGAG CGCGGTGGCA ATGCCCTGCC
6601 TCCGGTGCTC GCCGGAGACT GCGAGATCAT AGATATAGAT CTCACTACGC GGCTGCTCAA
6661 ACCTGGGCAG AACGTAAGCC GCGAGAGCGC CAACAACCGC TTCTTGGTCG AAGGCAGCAA
6721 GCGCGATGAA TGTCTTACTA CGGAGCAAGT TCCCGAGGTA ATCGGAGTCC GGCTGATGTT
6781 GGGAGTAGGT GGCTACGTCT CCGAACTCAC GACCGAAAAG ATCAAGAGCA GCGCGCATGG
6841 ATTGACTTG GTCAGGGCCG AGCCTACATG TGCGAATGAT GCCCATACTT GAGCCACCTA
6901 ACTTTGTTTT AGGGCGACTG CCCTGCTGCG TAACATCGTT GCTGCTGCGT AACATCGTTG
6961 CTGCTCCATA ACATCAAACA TCGACCCACG GCGTAACGCG CTTGCTGCTT GGATGCCCCG
7021 GGCATAGACT GTACAAAAAA ACAGTCATAA CAAGCCATGA AAACCG

FIGURE 40D

Figure 41A:

pDBT21

**2-Hybrid Vector with
DNA-Binding Domain**

ADH Promoter

700 ~~ttg gcg ctg tgc tat caa gta taa ata gac ctg caa tta tta atc ttt tgt~~
~~aac ggc gaa acg ata gtt cat att tat ctg gac gtt aat aat taq aaa aca~~

751 ~~ttc ctc gtc att gtt ctc gtt ccc ttt ctt cct tgt ttc ttt ttc tgc aca~~
~~aad gag cag taa caa gag caa ggg aaa gaa gga aca aag aaa aag acg tgt~~

802 ~~ata ttt caa gct ata cca agc ata caa tca act~~ cca agc ttg aag caa gcc
~~tat aaa gtt cga tat ggt tgc tat gtt agt tga~~ ggt tgc aac ttc gtt cgg

853 ~~tcc tga aag atg aag cta ctg tct tct atc gaa caa gca tgc gat att tgc~~
~~agg act ttc tac ttc gat gac aga aga tag ctt gtt cgt acg cta taa acg~~

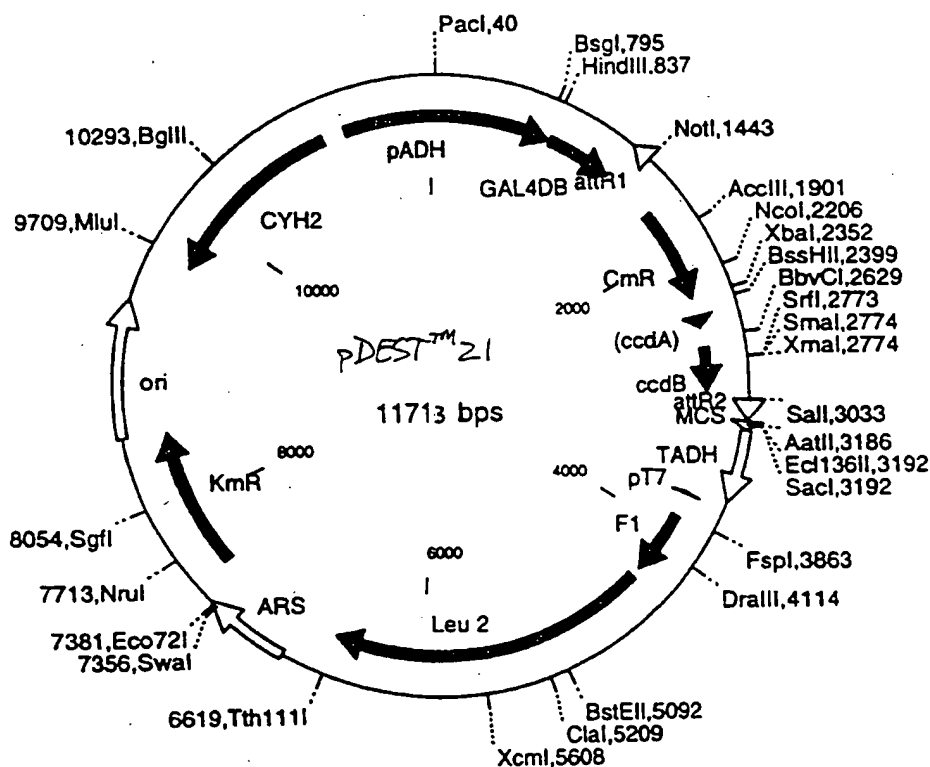
... gaa gag agt agt aac aaa ggt caa aga cag ttg act gta tgc tgc agg tgc
 ctt ctc tca tca ttg ttt cca gtt tct gtc aac tga cat agc agc tcc agc

1261 ~~aca agt ttg tac aaa aaa gct gaa cga gaa acg taa aat gat ata~~
~~tgt tca aac atg ttt ttt cga ctt gct ctt tgc att tta cta tat~~

1312

Start Transl. **M K L L S S - - Gal4-DB**

Int ↓



112/240

pDEST21 11713 bp (rotated to position 11000)

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
857..1322		GAL4DB
1456..1332		attR1
1706..2365		CmR
2485..2569		inactivated ccdA
2707..3012		ccdB
3053..3177		attR2
3716..3735		pT7 (T7 promoter)
3899..4354		f1 (f1 intergenic region)
4414..6642		Leu2
7541..8515		kanR
9668..10958		CYH2
11118..848		pADH (ADH promoter)

1	TTTATTATGT	TACAATATGG	AAGGGAACTT	TACACTTCTC	CTATGCACAT	ATATTAATTA
61	AAGTCCAATG	CTAGTAGAGA	AGGGGGGTAA	CACCCCTCCG	CGCTCTTTTC	CGATTTTTTT
121	CTAAACCGTG	GAATATTTTCG	GATATCCTTT	TGTTGTTTCC	GGGTGTACAA	TATGGACTTC
181	CTCTTTTCTG	GCAACCAAAC	CCATACATCG	GGATTCTCTAT	AATACCTTCG	TGGTCTCTCC
241	TAACATGTAG	GTGGCGGAGG	GGAGATATAC	AATAGAACAG	ATACCAGACA	AGACATAATG
301	GGCTAAACAA	GACTACACCA	ATTACACTGC	CTCATGTATG	GTGGTACATA	ACGAACTAAT
361	ACTGTAGCCC	TAGACTTGAT	AGCCATCATC	ATATCGAAGT	TTCCTACCCC	TTTTTCCATT
421	TGCCATCTAT	TGAAGTAATA	ATAGGCGCAT	GCAACTTCTT	TTCTTTTTTT	TTCTTTTCTC
481	TCTCCCCCGT	TGTTGTCTCA	CCATATCCGC	AATGACAAAA	AAAATGATGG	AAGACACTAA
541	AGGAAAAAAT	TAACGACAAA	GACAGCACCA	ACAGATGTCG	TTGTTCCAGA	GCTGATGAGG
601	GGTATCTTCG	AACACACGAA	ACTTTTTCCT	TCCTTCATTG	ACGCACACTA	CTCTCTAATG
661	AGCAACGGTA	TACGGCCTTC	CTTCCAGTTA	CTTGAATTTG	AAATAAAAAA	AGTTTGCCGC
721	TTTGCTATCA	AGTATAAATA	GACCTGCAAT	TATTAATCTT	TTGTTTCCTC	GTCATTGTTT
781	TCGTTCCCTT	TCTTCCTTGT	TTCTTTTCT	GCACAATATT	TCAAGCTATA	CCAAGCATAC
841	AATCAACTCC	AAGCTTGAAG	CAAGCCTCCT	GAAAGATGAA	GCTACTGTCT	TCTATCGAAC
901	AAGCATGCGA	TATTTGCCGA	CTTAAAAAGC	TCAAGTGCTC	CAAAGAAAAA	CCGAAGTGCG
961	CCAAGTGTCT	GAAGAACAAC	TGGGAGTGTC	GCTACTCTCC	CAAACCAAAA	AGGTCTCCGC
1021	TGACTAGGGC	ACATCTGACA	GAAGTGAAT	CAAGGCTAGA	AAGACTGGAA	CAGCTATTTT
1081	TACTGATTTT	TCCTCGAGAA	GACCTTGACA	TGATTTTGAA	AATGGATTCT	TTACAGGATA
1141	TAAAAGCATT	GTTAACAGGA	TTATTTGTAC	AAGATAATGT	GAATAAAGAT	GCCGTCACAG
1201	ATAGATTGGC	TTCAAGTGGG	ACTGATATGC	CTCTAACATT	GAGACAGCAT	AGAATAAGTG
1261	CGACATCATC	ATCGGAAGAG	AGTAGTAACA	AAGGTCAAAG	ACAGTTGACT	GTATCGTCGA
1321	GGTCGAATCA	AACAAGTTTG	TACAAAAAAG	CTGAACGAGA	AACGTAATAA	GATATAAATA
1381	TCAATATATT	AAATTAGATT	TTGCATAAAA	AACAGACTAC	ATAATACTGT	AAAACACAAC
1441	ATATCCAGTC	ACTATGGCGG	CCGCTAAGTT	GGCAGCATCA	CCCGACGCAC	TTTGCGCCGA
1501	ATAAATACCT	GTGACGGAAG	ATCACTTCGC	AGAATAAATA	AATCCTGGTG	TCCCTGTTGA
1561	TACCGGGAAG	CCCTGGGCCA	ACTTTTGGCG	AAAATGAGAC	GTTGATCGGC	ACGTAAGAGG
1621	TTCCAACCTT	CACCATAATG	AAATAAGATC	ACTACCGGGC	GTATTTTTTG	AGTTATCGAG
1681	ATTTTCAGGA	GCTAAGGAAG	CTAAATGGA	GAAAAAATC	ACTGGATATA	CCACCGTTGA
1741	TATATCCCAA	TGGCATCGTA	AAGAACATTT	TGAGGCATTT	CAGTCAGTTG	CTCAATGTAC
1801	CTATAACCAG	ACCGTTCAGC	TGGATATTAC	GGCCTTTTTA	AAGACCGTAA	AGAAAAATAA
1861	GCACAAGTTT	TATCCGGCCT	TTATTCACAT	TCTTGCCCGC	CTGATGAATG	CTCATCCGGA
1921	ATTCCGTATG	GCAATGAAAG	ACGGTGAGCT	GGTGATATGG	GATAGTGTTC	ACCCTTGTTA
1981	CACCGTTTTT	CATGAGCAAA	CTGAAACGTT	TTCATCGCTC	TGGAGTGAAT	ACCACGACGA
2041	TTTCCGGCAG	TTTCTACACA	TATATTCGCA	AGATGTGGCG	TGTTACGGTG	AAAACCTGGC
2101	CTATTTCCCT	AAAGGGTTTA	TTGAGAATAT	GTTTTTCGTC	TCAGCCAATC	CCTGGGTGAG
2161	TTTCAACAGT	TTTGATTTAA	ACGTGGCCAA	TATGGACAAC	TTCTTCGCCC	CCGTTTTTAC
2221	CATGGGCAAA	TATTATACGC	AAGGCGACAA	GGTGCTGATG	CCGCTGGCGA	TTCAAGTTCA
2281	TCATGCCGTC	TGTGATGGCT	TCCATGTCGG	CAGAATGCTT	AATGAATTAC	AACAGTACTG
2341	CGATGAGTGG	CAGGGCGGGG	CGTAATCTAG	AGGATCCGGC	TTACTAAAAG	CCAGATAACA
2401	GTATGCGTAT	TTGCGCGCTG	ATTTTTCGCG	TATAAGAATA	TATACTGATA	TGTATACCCG-

FIGURE 413

2461 AAGTATGTCA AAAAGAGGTG TGCTATGAAG CAGCGTATTA CAGTGACAGT TGACAGCGAC
2521 AGCTATCAGT TGCTCAAGGC ATATATGATG TCAATATCTC CGGTCTGGTA AGCACAACCA
2581 TGCAGAATGA AGCCCGTCGT CTGCGTGCCG AACGCTGGAA AGCGGAAAAT CAGGAAGGGA
2641 TGGCTGAGGT CGCCCGGTTT ATTGAAATGA ACGGCTCTTT TGCTGACGAG AACAGGGACT
2701 GGTGAAATGC AGTTTAAGGT TTACACCTAT AAAAGAGAGA GCCGTATATCG TCTGTTTGTG
2761 GATGTACAGA GTGATATTAT TGACACGCCC GGGCGACGGA TGGTGATCCC CCTGGCCAGT
2821 GCACGTCTGC TGTCAGATAA AGTCTCCCGT GAACTTTACC CGGTGGTGCA TATCGGGGAT
2881 GAAAGCTGGC GCATGATGAC CACCGATATG GCCAGTGTGC CGGTCTCCGT TATCGGGGAA
2941 GAAGTGGCTG ATCTCAGCCA CCGCGAAAAT GACATCAAAA ACGCCATTAA CCTGATGTTT
3001 TGGGGAATAT AAATGTCAGG CTCCCTTATA CACAGCCAGT CTGCAGGTCG ACCATAGTGA
3061 CTGGATATGT TGTGTTTTAC AGTATTATGT AGTCTGTTTT TTATGCAAAA TCTAATTTAA
3121 TATATTGATA TTTATATCAT TTTACGTTTC TCGTTCAGCT TTCTTGATCA AAGTGGTTTG
3181 ATGGCCGCTA AGTAAGTAAG ACGTCGAGCT CTAAGTAAGT AACGGCCGCC ACCGCGGTGG
3241 AGCTTTGGAC TTCTTCGCCA GAGGTTTGGT CAAGTCTCCA ATCAAGGTTG TCGGCTTGTC
3301 TACCTTGCCA GAAATTTACG AAAAGATGGA AAAGGGTCAA ATCGTTGGTA GATACGTTGT
3361 TGACACTTCT AAATAAGCGA ATTTCTTATG ATTTATGATT TTTATTATTA AATAAGTTAT
3421 AAAAAAATA AGTGATACA AATTTTAAAG TGACTCTTAG GTTTTAAAC GAAAATTCTT
3481 ATTCTTGAGT AACTCTTTCC TGTAGGTCAG GTTGCTTTCT CAGGTATAGC ATGAGGTGCG
3541 TCTTATTGAC CACACCTCTA CCGGCATGCC GAGCAAATGC CTGCAAATCG CTCCCCATTT
3601 CACCCAATTG TAGATATGCT AACTCCAGCA ATGAGTTGAT GAATCTCGGT GTGTATTTA
3661 TGTCCTCAGA GGACAATACC TGTGTAAATC GTTCTTCCAC ACGGATCCCA ATTCGCCCTA
3721 TAGTGAGTCG TATTACAATT CACTGGCCGT CGTTTTACAA CGTCGTGACT GGGAAAACCC
3781 TGGCGTTACC CAACTTAATC GCCTTGCAGC ACATCCCCCT TTCGCCAGCT GGCCTAATAG
3841 CGAAGAGGCC CGCACCGATC GCCCTTCCCA ACAGTTGCGC AGCCTGAATG GCGAATGGAC
3901 GCGCCCTGTA GCGGCGCATT AAGCGCGGCG GGTGTGGTGG TTACGCGCAG CGTGACCGCT
3961 ACACTTGCCA GCGCCCTAGC GCCCGCTCCT TTCGCTTTCT TCCCTTCCTT TCTCGCCACG
4021 TTCGCCGGCT TTCCCCGTCA AGCTCTAAAT CGGGGGCTCC CTTTAGGGTT CCGATTTAGT
4081 GCTTTACGGC ACCTCGACCC CAAAAAATT GATTAGGGTG ATGGTTCACG TAGTGGGCCA
4141 TCGCCCTGAT AGACGGTTTT TCGCCCTTTG ACGTTGGAGT CCACGTTCTT TAATAGTGGA
4201 CTCTTGTTCC AAACCTGGAAC AACACTCAAC CCTATCTCGG TCTATTCTTT TGATTTATAA
4261 GGGATTTTGC CGATTTCGGC CTATTGGTTA AAAAATGAGC TGATTTAACA AAAATTTAAC
4321 GCGAATTTTA ACAAATATT AACGTTTACA ATTTCTGAT GCGGTATTTT CTCCTTACGC
4381 ATCTGTGCGG TATTTCACAC CGCATATCGA CCGGTGCGAG AGAATTTCTA GTATATCCAC
4441 ATACCTAATA TTATTGCCTT ATTAAAAATG GAATCGGAAC AATTACATCA AAATCCACAT
4501 TCTCTTCAA ATCAATTGTC CTGTACTTCC TTGTTTATGT GTGTTCAAAA ACGTTATATT
4561 TATAGGATAA TTATACTCTA TTTCTCAACA AGTAATTGGT TGTGTTGGCCG AGCGGTCTAA
4621 GCGCCCTGAT TCAAGAAATA TCTTGACCGC AGTTAACTGT GGAATACTC AGGTATCGTA
4681 AGATGCAAGA GTTCGAATCT CTTAGCAACC ATTATTTTTT TCCTCAACAT AACGAGAACA
4741 CACAGGGGCG CTATCGCACA GAATCAAATT CGATGACTGG AAATTTTTTG TTAATTTTACG
4801 AGGTGCGCTG ACGCATATAC CTTTTTCAAC TGAAAAATTG GGAGAAAAAG GAAAGGTGAG
4861 AGGCCGGAAC CGGCTTTTCA TATAGAATAG AGAAGCGTTC ATGACTAAAT GCTTGCATCA
4921 CAATACTTGA AGTTGACAA ATTTATTAAAG GACCTATTGT TTTTCCAAAT AGGTGGTTAG
4981 CAATCGTCTT ACTTTCTAAC TTTTCTTACC TTTTACATTT CAGCAATATA TATATATATT
5041 TCAAGGATAT ACCATTCTAA TGTCTGCCCC TATGTCTGCC CTAAGAAGA TCGTCGTTTT
5101 GCCAGGTGAC CACGTTGGTC AAGAAATCAC AGCCGAAGCC ATTAAGGTTT TTAAGCTAT
5161 TTCTGATGTT CGTTCCAATG TCAAGTTCGA TTTGAAAAT CATTTAATTG GTGGTGCTGC
5221 TATCGATGCT ACAGGTGTCC CACTTCCAGA TGAGGCGCTG GAAGCCTCCA AGAAGGTTGA
5281 TGCCGTTTTG TTAGGTGCTG TGGGTGGTCC TAAATGGGGT ACCGGTAGTG TTAGACCTGA
5341 ACAAGGTTTA CTAAAAATCC GTAAAGAACT TCAATTGTAC GCCAACTTAA GACCATGTAA
5401 CTTTGCATCC GACTCTCTTT TAGACTTATC TCCAATCAAG CCACAATTTG CTAAAGGTAC
5461 TGACTTCGTT GTTGTCAGAG AATTAGTGGG AGGTATTTAC TTGGTAAGA GAAAGGAAGA
5521 CGATGGTGAT GGTGTCGCTT GGGATAGTGA ACAATACACC GTTCCAGAAG TGCAAAGAAT
5581 CACAAGAATG GCCGCTTTCA TGGCCCTACA ACATGAGCCA CCATTGCCTA TTTGGTCCTT
5641 GGATAAAGCT AATGTTTTGG CCTCTTCAAG ATTATGGAGA AAAACTGTGG AGGAAACCAT
5701 CAAGAACGAA TTCCCTACAT TGAAGGTTCA ACATCAATTG ATTGATTCTG CCGCCATGAT
5761 CCTAGTTAAG AACCCAACCC ACCTAAATGG TATTATAATC ACCAGCAACA TGTTTTGGTGA
5821 TATCATCTCC GATGAAGCCT CCGTTATCCC AGGTTCTTTG GGTGTTGTGC CATCTGCGTC
5881 CTTGGCCTCT TTGCCAGACA AGAACACCGC ATTTGGTTTG TACGAACCAT GCCACGGTTC

114/240

5941 TGCTCCAGAT TTGCCAAAGA ATAAGGTTGA CCCTATCGCC ACTATCTTGT CTGCTGCAAT
6001 GATGTTGAAA TTGTCATTGA ACTTGCCCTGA AGAAGGTAAG GCCATTGAAG ATGCAGTTAA
6061 AAAGGTTTTG GATGCAGGTA TCAGAACTGG TGATTTAGGT GGTTCACAAC GTACCACCGA
6121 AGTCGGTGAT GCTGTCGCCG AAGAAGTTAA GAAAATCCTT GCTTAAAAAG ATTCTCTTTT
6181 TTTATGATAT TTGTACATAA ACTTTATATA TGAAATTCAT AATAGAAACG ACACGAAATT
6241 ACAAATGGA ATATGTTTCA AGGGTAGACG AAACATATATA CGCAATCTAC ATACATTTAT
6301 CAAGAAGGAG AAAAAGGAGG ATAGTAAAGG AATACAGGTA AGCAAATTGA TACTAATGGC
6361 TCAACGTGAT AAGGAAAAAG AATTGCACCT TAACATTAAT ATTGACAAGG AGGAGGGCAC
6421 CACACAAAAA GTTAGGTGTA ACAGAAAAATC ATGAAACTAC GATTCCTAAT TTGATATTGG
6481 AGGATTTTCT CTAAAAAATA AAAAATACAA CAAATAAAAA AACTCAATG ACCTGACCAT
6541 TTGATGGAGT TTAAGTCAAT ACCTTCTTGA ACCATTTCCC ATAATGGTGA AAGTTCCCTC
6601 AAGAATTTTA CTCTGTGAGA AACGGCCTTA CGACGTAGTC GATATGGTGC ACTCTCAGTA
6661 CAATCTGCTC TGATGCCGCA TAGTTAAGCC AGCCCCGACA CCCGCCAACA CCCGCTGACG
6721 CGCCCTGACG GGCTTGTCTG CTCCCGGCAT CCGCTTACAG ACAAGCTGTG ACCGTCTCCG
6781 GGAGCTGCAT GTGTCAGAGG TTTTACCCTG CATCACCGAA ACGCGCGAGA CGAAAGGGCC
6841 TCGTGATACG CCTATTTTTA TAGGTTAATG TCATGATAAT AATGGTTTCT TAGGACGGAT
6901 CGCTTGCCCTG TAACCTACAC GCGCCTCGTA TCTTTTAATG ATGGAATAAT TTGGGAATTT
6961 ACTCTGTGTT TATTTATTTT TATGTTTTGT ATTTGGATTT TAGAAAAGTAA ATAAAGAAGG
7021 TAGAAGAGTT ACGGAATGAA GAAAAAATA TAAACAAAGG TTTAAAAAAT TTCAACAAAA
7081 AGCGTAGCTT ACATATATAT TTATTAGACA AGAAAAGCAG ATTAAATAGA TATACATTCG
7141 ATTAACGATA AGTAAATGT AAAATCACAG GATTTTCGTG TGTGGTCTTC TACACAGACA
7201 AGATGAAACA ATTCGGCATT AATACCTGAG AGCAGGAAGA GCAAGATAAA AGGTAGTATT
7261 TGTGGCGAT CCCCTAGAG TCTTTTACAT CTTCGGAAAA CAAAAACTAT TTTTCTTTA
7321 ATTTCTTTTT TACTTTCTA TTTTAAATTT ATATATTTAT ATTAAAAAAT TTAAATTATA
7381 ATTATTTTTA TAGCACGTGA TGAAAAGGAC CCAGGTGGCA CTTTTCGGGG AAATGTGCGC
7441 GGAACCCCTA TTTGTTTATT TTTCTAAATA CATTCAAATA TGTATCCGCT CATGAGACAA
7501 TAACCTGAT AAATGCTTCA ATAATCTGCA GCTCTGGCCC GTGTCTCAA ATCTCTGATG
7561 TTACATTGCA CAAGATAAAA ATATATCATC ATGAACAATA AAACGTGCTG CTTACATAAA
7621 CAGTAATACA AGGGGTGTTA TGAGCCATAT TCAACGGGAA ACGTCTTGCT GGAGGCCGCG
7681 ATTAAATTCC AACATGGATG CTGATTTATA TGGGTATAAA TGGGCTCGCG ATAATGTCGG
7741 GCAATCAGGT GCGACAATCT TTCGATTGTA TGGGAAGCCC GATGCGCCAG AGTTGTTTCT
7801 GAAACATGGC AAAGGTAGCG TTGCCAATGA TGTTACAGAT GAGATGGTCA GACTAAACTG
7861 GCTGACGGAA TTTATGCCTC TTCCGACCAT CAAGCATTTT ATCCGTACTC CTGATGATGC
7921 ATGGTTACTC ACCACTGCGA TCCGCGGGAA AACAGCATTC CAGGTATTAG AAGAATATCC
7981 TGATTCAGGT GAAAATATTG TTGATGCGCT GGCAGTGTTT CTGCGCCGGT TGCATTTCGAT
8041 TCCTGTTTGT AATTGTCCTT TTAACAGCGA TCGCGTATTT CGTCTCGCTC AGCGCAATC
8101 ACGAATGAAT AACGGTTTGG TTGATGCGAG TGATTTTGAT GACGAGCGTA ATGGCTGGCC
8161 TGTTGAACAA GTCTGGAAAG AAATGCATAC GCTTTTGCCA TTCTCACCAG ATTCACTCGT
8221 CACTCATGGT GATTTCTCAC TTGATAACCT TATTTTGTAC GAGGGGAAAT TAATAGGTTG
8281 TATTGATGTT GGACGAGTCG GAATCGCAGA CCGATACCAG GATCTTGCCA TCCTATGGAA
8341 CTGCTCGGT GAGTTTCTC CTTCATTACA GAAACGGCTT TTTCAAAAAT ATGGTATTGA
8401 TAATCCTGAT ATGAATAAAT TGCAGTTTCA TTTGATGCTC GATGAGTTT TCTAATCAGA
8461 ATTGGTTAAT TGGTTGTAAC ACTGGCAGAG CATTACGCTG ACTTGACGGG ACGGCGCATG
8521 ACCAAAATCC CTTAACGTGA GTTTTCGTTC CACTGAGCGT CAGACCCCGT AGAAAAGATC
8581 AAAGGATCTT CTTGAGATCC TTTTCTCTG CCGTAATCT GCTGCTTGCA AACAAAAAA
8641 CCACCGCTAC CAGCGGTGGT TTGTTTGCCG GATCAAGAGC TACCAACTCT TTTTCCGAAG
8701 GTAACCTGGT TCAGCAGAGC GCAGATACCA AATACTGTCC TTCTAGTGTA GCCGTAGTTA
8761 GGCCACCACT TCAAGAACTC TGTAGCACCG CCTACATACC TCGCTCTGCT AATCCTGTTA
8821 CCAGTGGCTG CTGCCAGTGG CGATAAGTCG TGTCTTACC GGTGGACTC AAGACGATAG
8881 TTACCGGATA AGGCGCAGCG GTCCGGGTGA ACGGGGGGTT CGTGACACA GCCCAGCTTG
8941 GAGCGAACGA CCTACACCGA ACTGAGATAC CTACAGCGTG AGCATTGAGA AAGCGCCAG
9001 CTTCCCGAAG GGAGAAAGGC GGACAGGTAT CCGGTAAGCG GCAGGGTCGG AACAGGAGAG
9061 CGCACGAGGG AGCTTCCAGG GGGGAACGCC TGGTATCTTT ATAGTCCTGT CGGGTTTCGC
9121 CACCTCTGAC TTGAGCGTCG ATTTTGTGTA TGCTCGTCAG GGGGGCCGAG CCTATGGAAA
9181 AACGCCAGCA ACGCGGCCTT TTTACGGTTC CTGGCCTTTT GCTGGCCTTT TGCTCACATG
9241 TTCTTTCTCT CGTTATCCCC TGATTCTGTG GATAACCGTA TTACCGCCTT TGAGTGAGCT
9301 GATACCGCTC GCCGACCGG AACGACCGAG CGCAGCGAGT CAGTGAGCGA GGAAGCGGAA
9361 GAGCGCCCAA TACGCAAACC GCCTCTCCCC GCGGTTGGC CGATTCTTA ATGCAGCTGG

FIGURE 4LD

115/240

9421 CACGACAGGT TTCCCGACTG GAAAGCGGGC AGTGAGCGCA ACGCAATTAA TGTGAGTTAC
9481 CTCACCTCATT AGGCACCCCA GGCTTTACAC TTTATGCTTC CGGCTCCTAT GTTGTGTGGA
9541 ATTGTGAGCG GATAACAATT TCACACAGGA AACAGCTATG ACCATGATTA CGCCAAGCTC
9601 GGAATTAACC CTCACTAAAG GGAACAAAAG CTGGTACCGA TCCCGAGCTT TGCAAATTAA
9661 AGCCTTCGAG CGTCCCAAAA CCTTCTCAAG CAAGGTTTTT AGTATAATGT TACATGCGTA
9721 CACGCGTCTG TACAGAAAAA AAAGAAAAAT TTGAAATATA AATAACGTTT TTAATACTAA
9781 CATAACTATA AAAAAATAAA TAGGGACCTA GACTTCAGGT TGTCTAACTC CTCCTTTTTT
9841 GGTTAGAGCG GATGTGGGGG GAGGGCGTGA ATGTAAGCGT GACATAACTA ATTACATGAT
9901 ATCGACAAAG GAAAAGGGGC CTGTTTACTC ACAGGCTTTT TTCAGTAGG TAATTAAGTC
9961 GTTTCGTGCT TTTTCCTTCT TCAACCCACC AAAGGCCATC TTGGTACTTT TTTTTTTTTT
10021 TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT
10081 TTTTTTTTTT TTTTTTTTTT TCATAGAAAT AATACAGAAG TAGATGTTGA ATTAGATTAA
10141 ACTGAAGATA TATAATTAT TGGAAAATAC ATAGAGCTTT TTGTTGATGC GCTTAAGCGA
10201 TCAATTCAAC AACACCACCA GCAGCTCTGA TTTTTCTTC AGCCAACTTG GAGACGAATC
10261 TAGCTTTGAC GATAACTGGA ACATTTGGAA TTCTACCTT ACCCAAGATC TTACCGTAAC
10321 CGGCTGCCAA AGTGTCATA ACTGGAGCAG TTTCTTAGA AGCAGATTTC AAGTATTGGT
10381 CTCTCTGTG TCCTGGGATC AATGTCCACA ATTTGTCCAA GTTCAAGACT GGCTTCCAGA
10441 AATGAGCTTG TTGCTTGTG AAGTATCTCA TACCAACCTT ACCGAAATAA CCTGGATGGT
10501 ATTTATCCAT GTTAATTCTG TGGTGATGTT GACCACCGGC CATACCTCTA CCACCGGGT
10561 GCTTCTGTG CTTACCGATA CGACCTTTAC CGGCTGAGAC GTGACCTCTG TGCTTCTAG
10621 TCTTAGTGAA TCTGGAAGGC ATTCTTGATT AGTTGGATGA TTGTTCTGGG ATTTAATGCA
10681 AAAATCACTT AAGAAGGAAA ATCAACGGAG AAAGCAAACG CCATCTTAAA TATACGGGAT
10741 ACAGATGAAA GGGTTTGAAC CTATCTGGAA AATAGCATT AACAAGCGAA AACTGCGAG
10801 GAAAATTGTT TGCCTCTCTG CGGCTATTC ACGCGCCAGA GGAAAATAGG AAAAAATAA
10861 GGGCATTAGA AAAATAATTT TGATTTTGGT AATGTGTGGG TCCTGGTGTA CAGATGTTAC
10921 ATTGGTTACA GTACTCTTGT TTTTGCTGTG TTTTTCGATG AATCTCCAAA ATGGTTGTTA
10981 GCACATGGAA GAGTCACCGA TGCTAAGTTA TCTCTATGTA AGCTACGTGG CGTGACTTTT
11041 GATGAAGCCG CACAAGAGAT ACAGGATTGG CAACTGCAAA TAGAATCTGG GGATCCCCC
11101 TCGAGATCCG GGATCGAAGA AATGATGGTA AATGAAATAG GAAATCAAGG AGCATGAAGG
11161 CAAAAGACAA ATATAAGGGT CGAACGAAAA ATAAAGTGAA AAGTGTGAT ATGATGTATT
11221 TGGCTTTGCG GCGCCGAAAA AACGAGTTTA CGCAATTGCA CAATCATGCT GACTCTGTGG
11281 CGGACCCGCG CTCTTGCCGG CCCGGCGATA ACGCTGGGCG TGAGGCTGTG CCCGGCGGAG
11341 TTTTTTGCGC CTGCATTTTC CAAGGTTTAC CCTGCGCTAA GGGGCGAGAT TGGAGAAGCA
11401 ATAAGAATGC CGGTTGGGGT TGCGATGATG ACGACCACGA CAACTGGTGT CATTATTAA
11461 GTTGCCGAAA GAACCTGAGT GCATTTGCAA CATGAGTATA CTAGAAGAAT GAGCCAAGAC
11521 TTGCGAGACG CGAGTTTGCC GGTGGTGCGA ACAATAGAGC GACCATGACC TTGAAGGTGA
11581 GACGCGCATA ACCGCTAGAG TACTTTGAAG AGGAAACAGC AATAGGGTTG CTACCAGTAT
11641 AAATAGACAG GTACATACAA CACTGGAAAT GGTGTCTGT TTGAGTACGC TTTCAATTCA
11701 TTTGGGTGTG CAC

FIGURE 415

Figure 42A:

pDEST22

2-Hybrid Vector with
Activation Domain

657 acg cac act act ctc taa tga gca acg gta tac ggc ctt cct tcc agt tac
tgc gtg tga tga gag att act cgt tgc cat atg ccg gaa gga agg tca atg

708 ttg aat ttg aaa taa aaa aag ttt gcc gct ttg cta tca agt ata aat aga
aac tta aac ttt att ttt ttc aaa cgg cga aac gat agt tca tat tta tct

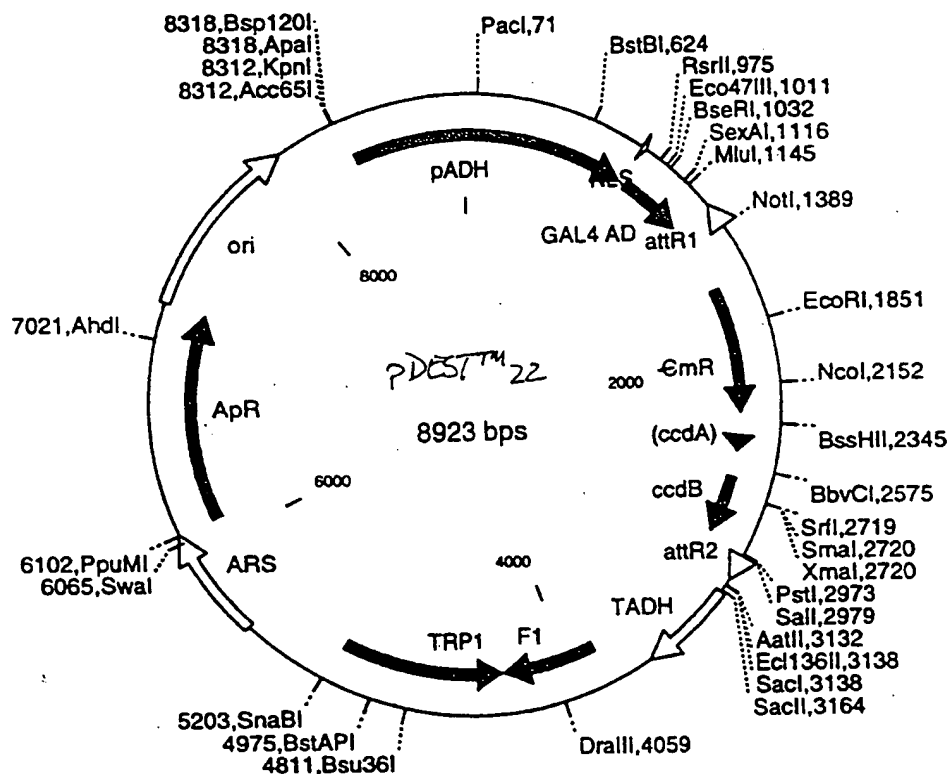
759 cct gca att att aat ctt ttg ttt cct cgt cat tgt tct cgt tcc ctt tct
gga cgt taa taa tta gaa aac aaa gga gca gta aca aga gca agg gaa aga

810 *ADH Promoter*
"ccc ttg ttt ctt ttt gtc cat aat att tca agc tat acc aag cat aca atc"
"agg aac aaa gga aaa gac gtg cta taa agt tgc ata tgg ttc gta tat tag"

861 *Gal4-AD*
"aac tcc aag ctt atg ccc aag aag aag cgg aag gtc tgc agc ggc gcc aat"
"ttg agg ttc gaa tac ggg ttc ttc ttc gcc ttc cag agc tgc ccg cgg ttg"
Start Translation

1218 gaa gat acc cca cca aac cca aaa aaa gag ggt ggg tgg aat caa aca agt
ctt cta tgg ggt ggt ttg ggt ttt ttt ctc cca ccc agc tta gtt tgt tca

1269 *LI Y K K A attR1*
"ttg tac aaa aaa gct gaa cga gaa acg taa a"
"aac atg ttt ttt cga ctt gct ctt tgc att t"
IntV



117/240

pDEST22 8923 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
904..1248		GAL4 AD
1388..1264		attR1
1638..2297		CmR
2417..2501		inactivated ccdA
2639..2944		ccdB
2985..3109		attR2
3831..4318		f1 (f1 intergenic region)
4334..5176		TRP1
6110..7194		ampR
8344..866		pADH (yeast ADH promoter)

1	TTCATTTGGG	TGTGCACTTT	ATTATGTTAC	AATATGGAAG	GGAACCTTTAC	ACTTCTCCTA
61	TGCACATATA	TTAATTAAAG	TCCAATGCTA	GTAGAGAAGG	GGGGTAACAC	CCCTCCGCGC
121	TCTTTTCCGA	TTTTTTTCTA	AACCGTGGA	TATTTCCGAT	ATCCTTTTGT	TGTTTCCGGG
181	TGTACAATAT	GGACTTCCTC	TTTTCTGGCA	ACCAAACCCA	TACATCGGGA	TTCCTATAAT
241	ACCTTCGTTG	GTCTCCCTAA	CATGTAGGTG	GCGGAGGGGA	GATATACAAT	AGAACAGATA
301	CCAGACAAGA	CATAATGGGC	TAAACAAGAC	TACACCAATT	ACACTGCCTC	ATTGATGGTG
361	GTACATAACG	AACTAATACT	GTAGCCCTAG	ACTTGATAGC	CATCATCATA	TCGAAGTTTC
421	ACTACCCTTT	TTCCATTGTC	CATCTATTGA	AGTAATAATA	GGCGCATGCA	ACTTCTTTTC
481	TTTTTTTTTC	TTTTCTCTCT	CCCCCGTTGT	TGTCTCACC	TATCCGCAAT	GACAAAAAAA
541	ATGATGGAAG	ACACTAAAGG	AAAAAATTAA	CGACAAAGAC	AGCACCAACA	GATGTCGTTG
601	TTCCAGAGCT	GATGAGGGGT	ATCTTCGAAC	ACACGAAACT	TTTTCTTCC	TTCATTACAG
661	CACACTACTC	TCTAATGAGC	AACGGTATAC	GGCCTTCCTT	CCAGTTACTT	GAATTTGAAA
721	TAAAAAAGT	TTGCCGCTTT	GCTATCAAGT	ATAAATAGAC	CTGCAATTAT	TAATCTTTTG
781	TTTCCTCGTC	ATTGTTCTCG	TTCCCTTTCT	TCCTTGTTTC	TTTTTCTGCA	CAATATTTC
841	AGCTATAACCA	AGCATACAAT	CAACTCCAAG	CTTATGCCCA	AGAAGAAGCG	GAAGGTCTCG
901	AGCGGCGCCA	ATTTTAATCA	AAGTGGGAAT	ATTGCTGATA	GCTCATTGTC	CTTCACTTTC
961	ACTAACAGTA	GCAACGGTCC	GAACCTCATA	ACAACCTCAA	CAAATTCTCA	AGCGCTTTCA
1021	CAACCAATTG	CCTCCTCTAA	CGTTTCATGAT	AACTTCATGA	ATAATGAAAT	CACGGCTAGT
1081	AAAATTGATG	ATGGTAATAA	TTCAAAACCA	CTGTACCTG	GTGGACGGA	CCAAACTGCG
1141	TATAACGCGT	TTGGAATCAC	TACAGGGATG	TTAATACCA	CTACAATGGA	TGATGTATAT
1201	AACTATCTAT	TCGATGATGA	AGATACCCCA	CCAAACCCAA	AAAAAGAGGG	TGGGTGCAAT
1261	CAAACAAGTT	TGTACAAAAA	AGCTGAACGA	GAAACGTAAA	ATGATATAAA	TATCAATATA
1321	TTAAATTAGA	TTTTGCATAA	AAAACAGACT	ACATAATACT	GTAAAAACACA	ACATATCCAG
1381	TCACTATGGC	GGCCGCTAAG	TTGGCAGCAT	CACCCGACGC	ACTTTGCGCC	GAATAAATAC
1441	CTGTGACGGA	AGATCACTTC	GCAGAATAAA	TAAATCCTGG	TGTCCCTGTT	GATACCGGGA
1501	AGCCCTGGGC	CAACTTTTGG	CGAAAATGAG	ACGTGATCG	GCACGTAAGA	GGTTCCAAC
1561	TTCAACCATA	TGAAATAAGA	TCACTACCGG	GCGTATTTTT	TGAGTTATCG	AGATTTTCAG
1621	GAGCTAAGGA	AGCTAAAATG	GAGAAAAAAA	TCACTGGATA	TACCACCGTT	GATATATCCC
1681	AATGGCATCG	TAAAGAACAT	TTTGAGGCAT	TTCACTCAGT	TGCTCAATGT	ACCTATAACC
1741	AGACCGTTCA	GCTGGATATT	ACGGCCTTTT	TAAAGACCGT	AAAGAAAAAT	AAGCACAAAGT
1801	TTTATCCGGC	CTTTATTTCAC	ATTCTTGCCC	GCCTGATGAA	TGCTCATCCG	GAATTCCGTA
1861	TGGCAATGAA	AGACGGTGAG	CTGGTGATAT	GGGATAGTGT	TCACCCCTGT	TACACCGTTT
1921	TCCATGAGCA	AACTGAAACG	TTTTTCATCGC	TCTGGAGTGA	ATACCACGAC	GATTTCCGGC
1981	AGTTTCTACA	CATATATTCC	CAAGATGTGG	CGTGTACGG	TGAAAACCTG	GCCTATTTCC
2041	CTAAAGGGTT	TATTGAGAAT	ATGTTTTTCG	TCTCAGCCAA	TCCCTGGGTG	AGTTTCACCA
2101	GTTTGTGATT	AAACGTGGCC	AATATGGACA	ACTTCTTCGC	CCCCGTTTTT	ACCATGGGCA
2161	AATATTATAC	GCAAGGCGAC	AGGTCGTGTA	TGCCGCTGGC	GATTCAGGTT	CATCATCCCG
2221	TCTGTGATGG	CTTCCATGTC	GGCAGAATGC	TTAATGAATT	ACAACAGTAC	TGCGATGAGT
2281	GGCAGGGCGG	GGCGTAATCT	AGAGGATCCG	GCTTACTAAA	AGCCAGATAA	CAGTATGCGT
2341	ATTTCGCGCG	TGATTTTTGC	GGTATAAGAA	TATATACTGA	TATGTATACC	CGAAGTATGT
2401	CAAAAAGAGG	TGTGCTATGA	AGCAGCGTAT	TACAGTGACA	GTTGACAGCG	ACAGCTATCA
2461	GTTGCTCAAG	GCATATATGA	TGTCAATATC	TCCGGTCTGG	TAAGCACAAC	CATGCAGAA
2521	GAAGCCCGTC	GTCTGCGTGC	CGAACGCTGG	AAAGCGGAAA	ATCAGGAAGG	GATGGCTGAG-

FIGURE 42B

2581 GTCGCCCCGT TTATTGAAAT GAACGGCTCT TTTGCTGACG AGAACAGGGA CTGGTGAAT
2641 GCAGTTTAAAG GTTTACACCT ATAAAAGAGA GAGCCGTTAT CGTCTGTTT TGGATGTACA
2701 GAGTGATATT ATTGACACGC CCGGGCGACG GATGGTGATC CCCCTGGCCA GTGCACGTCT
2761 GCTGTCAGAT AAAGTCTCCC GTGAACTTTA CCCGGTGGTG CATATCGGG ATGAAAGCTG
2821 GCGCATGATG ACCACCGATA TGGCCAGTGT GCCGGTCTCC GTTATCGGG AAGAAGTGCG
2881 TGATCTCAGC CACCGCGAAA ATGACATCAA AAACGCCATT AACCTGATGT TCTGGGGAAT
2941 ATAAATGTCA GGCTCCCTTA TACACAGCCA GTCTGCAGGT CGACCATAGT GACTGGATAT
3001 GTTGTGTTTT ACAGTATTAT GTAGTCTGTT TTTTATGCAA AATCTAATTT AATATATTGA
3061 TATTTATATC ATTTTACGTT TCTCGTTCAG CTTTCTTGTA CAAAGTGGTT TGATGGCCGC
3121 TAAGTAAGTA AGACGTCGAG CTCTAAGTAA GTAACGGCCG CCACCGCGGT GGAGCTTTGG
3181 ACTTCTTCGC CAGAGGTTTG GTCAAGTCTC CAATCAAGGT TGTCGGCTTG TCTACCTTGC
3241 CAGAAATTTA CGAAAAGATG GAAAAGGGTC AAATCGTTGG TAGATACGTT GTTGACACTT
3301 CTAAATAAGC GAATTTCTTA TGATTTATGA TTTTATTAT TAAATAAGTT ATAAAAAAA
3361 TAAGTGTATA CAAATTTTAA AGTGACTCTT AGGTTTTAAA ACGAAAATTC TTATCTTTGA
3421 GTAACCTCTT CCTGTAGGTC AGGTTGCTTT CTCAGGTATA GCATGAGGTC GCTCTTATG
3481 ACCACACCTC TACCGGCATG CCGAGCAAAT GCCTGCAAAT CGCTCCCAT TTCACCCAAT
3541 TGTAGATATG CTAACCTCAG CAATGAGTTG ATGAATCTCG GTGTGTATTT TATGTCCTCA
3601 GAGGACAATA CCTGTTGTAA TCGTTCTTCC ACACGGATCC CAATTCGCCC TATAGTGAGT
3661 CGTATTACAA TTCACTGGCC GTCGTTTTAC AACGTCGTGA CTGGGAAAAC CCTGGCGTTA
3721 CCCAACTTAA TCGCCTTGCA GCACATCCCC CTTTCGCCAG CTGGCGTAAT AGCGAAGAGG
3781 CCCGCACCGA TCGCCCTTCC CAACAGTTGC GCAGCCTGAA TGGCGAATGG ACGCGCCCTG
3841 TAGCGGCGCA TTAAGCGCGG CGGGTGTGGT GGTACGCGC AGCGTGACCG CTACACTTGC
3901 CAGCGCCCTA GCGCCCGCTC CTTTCGCTTT CTTCCCTTCC TTTCTCGCCA CGTTCGCGG
3961 CTTTCCCCGT CAAGCTCTAA ATCGGGGGCT CCCTTAGGG TTCCGATTTA GTGCTTTACG
4021 GCACCTCGAC CCCAAAAAAC TTGATTAGGG TGATGGTTCA CGTAGTGGG CATCGCCCTG
4081 ATAGACGGTT TTTGCCCCCT TGACGTTGGA GTCCACGTT TTAATAGTG GACTCTTGTT
4141 CCAAACTGGA ACAACACTCA ACCCTATCTC GGTCTATTCT TTTGATTTAT AAGGGATTTT
4201 GCCGATTTG CCCTATTGGT TAAAAATGA GCTGATTTAA CAAAAATTTA ACGCGAATTT
4261 TAACAAAATA TTAACGTTTA CAATTTCTCG ATGCGGTATT TTCTCCTTAC GCATCTGTGC
4321 GGTATTTTCA ACCGCAGGCA AGTGCACAAA CAATACTTAA ATAAATACTA CTCAGTAATA
4381 ACCTATTTCT TAGCATTTTT GACGAAATTT GCTATTTTGT TAGAGTCTTT TACACATTT
4441 GTCTCCACAC CTCCGCTTAC ATCAACACCA ATAACGCCAT TTAATCTAAG CGCATCACCA
4501 ACATTTTCTG GCGTCAGTCC ACCAGCTAAC ATAAATGTA AGCTTTCGGG GCTCTCTTGC
4561 CTTCCAACCC AGTCAGAAAT CGAGTTCCAA TCCAAAAGTT CACCTGTCCC ACCTGCTTCT
4621 GAATCAAACA AGGGAATAAA CGAATGAGGT TTCTGTGAAG CTGCACTGAG TAGTATGTTG
4681 CAGTCTTTTG GAAATACGAG TCTTTTAATA ACTGGCAAAC CGAGGAACTC TTGGTATTCT
4741 TGCCACGACT CATCTCCATG CAGTTGGACG ATATCAATGC CGTAATCATT GACCAGGCC
4801 AAAAATCCT CTTAGGTTG ATTACGAAAC ACGCCAACCA AGTATTTGCG AGTGCCGTGA
4861 CTATTTTTAT ATGCTTTTAC AAGACTTGAA ATTTTCTTGG CAATAACCGG GTCAATTGTT
4921 CTCTTTCTAT TGGGCACACA TATAATACCC AGCAAGTCAG CATCGGAATC TAGAGCACAT
4981 TCTGCGGCT CTGTGCTCTG CAAGCCGCAA ACTTTCACCA ATGGACCAGA ACTACCTGTG
5041 AAATTAATAA CAGACATACT CCAAGCTGCC TTTGTGTGCT TAATCACGTA TACTCACGTG
5101 CTCAATAGTC ACCAATGCCC TCCCTCTTGG CCCTCTCTT TTCTTTTTTC GACCGAATTA
5161 ATTCTTAATC GGCAAAAAA GAAAAGCTCC GGATCAAGAT TGTACGTAAG GTGACAAGCT
5221 ATTTTTCAAT AAAGAATATC TTCCACTACT GCCATCTGGC GTCATAACTG CAAAGTACAC
5281 ATATATTACG ATGCTGTCTA TTAAATGCTT CCTATATTAT ATATATAGTA ATGTCGTTTA
5341 TGGTGCACTC TCAGTACAAT CTGCTCTGAT GCCGCATAGT TAAGCCAGCC CCGACACCCG
5401 CCAACACCCG CTGACGCGCC CTGACGGGCT TGTCTGCTCC CGGCATCCGC TTACAGACAA
5461 GCTGTGACCG TCTCCGGGAG CTGCATGTGT CAGAGGTTTT CACCGTCATC ACCGAAACGC
5521 GCGAGACGAA AGGGCCTCGT GATACGCCTA TTTTATAGG TTAATGTCAT GATAATAATG
5581 GTTTCTTAGG ACGGATCGCT TGCCTGTAAC TTACACGCGC CTCGTATCTT TTAATGATGG
5641 AATAATTTGG GAATTTACTC TGTGTTTATT TATTTTATG TTTTGTATTT GGATTTTAGA
5701 AAGTAATAA AGAAGGTAGA AGAGTTACGG AATGAAGAAA AAAAAATAA CAAAGGTTTA
5761 AAAAAATTTCA ACAAAAAGCG TACTTTACAT ATATATTTAT TAGACAAGAA AAGCAGATTA
5821 AATAGATATA CATTCGATTA ACGATAAGTA AAATGTAAAA TCACAGGATT TCGTGTGTG
5881 GTCTTCTACA CAGACAAGAT GAAACAATTC GGCATTAATA CCTGAGAGCA GGAAGAGCAA
5941 GATAAAAGGT AGTATTGTG GCGCATCCCC CTAGAGTCTT TTACATCTTC GGAAACAAA
6001 AACTATTTTT TCTTTAATTT CTTTTTTTAC TTTCTATTTT TAATTTATAT ATTTATATTA-

FIGURE 42C

119/240

6061 AAAAATTTAA ATTATAATTA TTTTATAGC ACGTGATGAA AAGGACCCAG GTGGCACTTT
6121 TCGGGGAAAT GTGCGCGGAA CCCCTATTTG TTTATTTTTC TAAATACATT CAAATATGTA
6181 TCCGCTCATG AGACAATAAC CCTGATAAAT GCTTCAATAA TATTGAAAAA GGAAGAGTAT
6241 GAGTATTCAA CATTTCCTGTG TCGCCCTTAT TCCCTTTTTT GCGGCATTTT GCCTTCCTGT
6301 TTTTGCTCAC CCAGAAACGC TGGTGAAAGT AAAAGATGCT GAAGATCAGT TGGGTGCACG
6361 AGTGGGTTAC ATCGAACTGG ATCTCAACAG CGGTAAGATC CTTGAGAGTT TTCGCCCCGA
6421 AGAACGTTTT CCAATGATGA GCACTTTTAA AGTTCTGCTA TGTGGCGCGG TATTATCCCG
6481 TATTGACGCC GGGCAAGAGC AACTCGGTG CCGCATACAC TATTCTCAGA ATGACTTGGT
6541 TGAGTACTCA CCAGTCACAG AAAAGCATCT TACGGATGGC ATGACAGTAA GAGAATTATG
6601 CAGTGCTGCC ATAACCATGA GTGATAACAC TGCGGCCAAC TTAATTCTGA CAACGATCGG
6661 AGGACCGAAG GAGCTAACCG CTTTTTTTCA CAACATGGGG GATCATGTAA CTCGCCTTGA
6721 TCGTTGGGAA CCGGAGCTGA ATGAAGCCAT ACCAAACGAC GAGCGTGACA CCACGATGCC
6781 TGTAGCAATG GCAACAACGT TGCGCAAATC ATTAAGTGGC GAACTACTTA CTCTAGCTTC
6841 CCGGCAACAA TTAATAGACT GGATGGAGGC GGATAAAGTT GCAGGACCAC TTCTGCGCTC
6901 GGCCCTTCCG GCTGGCTGGT TTATTGCTGA TAAATCTGGA GCCGGTGAGC GTGGGTCTCG
6961 CGGTATCATT GCAGCACTGG GGCCAGATGG TAAGCCCTCC CGTATCGTAG TTATCTACAC
7021 GACGGGCAGT CAGGCAACTA TGGATGAACG AAATAGACAG ATCGCTGAGA TAGGTGCCTC
7081 ACTGATTAAG CATTGGTAAC TGTCAGACCA AGTTTACTCA TATATACTTT AGATTGATTT
7141 AAAACTTCAT TTTTAATTTA AAAGGATCTA GGTGAAGATC CTTTTTGATA ATCTCATGAC
7201 CAAAATCCCT TAACGTGAGT TTTCGTTCCA CTGAGCGTCA GACCCCGTAG AAAAGATCAA
7261 AGGATCTTCT TGAGATCCTT TTTTCTGCG CGTAATCTGC TGCTTGCAAA CAAAAAACC
7321 ACCGCTACCA GCGGTGGTTT GTTTGCCGGA TCAAGAGCTA CCAACTCTTT TTCCGAAGGT
7381 AAGTGGCTTC AGCAGAGCGC AGATACCAA TACTGTCCTT CTAGTGTAGC CGTAGTTAGG
7441 CCACCACTTC AAGAACTCTG TAGCACCGCC TACATACCTC GCTCTGCTAA TCCTGTTACC
7501 AGTGGCTGCT GCCAGTGGCG ATAAGTCGTG TCTTACCGGG TTGGACTCAA GACGATAGTT
7561 ACCGGATAAG GCGCAGCGGT CGGGCTGAAC GGGGGGTTTCG TGCACACAGC CCAGCTTGGA
7621 GCGAACGACC TACACCGAAC TGAGATACCT ACAGCGTGAG CATTGAGAAA GCGCCACGCT
7681 TCCCGAAGGG AGAAAGCGCG ACAGGTATCC GGTAAGCGGC AGGGTCGGAA CAGGAGAGCG
7741 CACGAGGGAG CTTCCAGGGG GGAACGCCTG GTATCTTTAT AGTCCTGTCT GGTTCGCCA
7801 CCTCTGACTT GAGCGTCGAT TTTTGTGATG CTCGTAGGG GGGCCGAGCC TATGGAAAAA
7861 CGCCAGCAAC GCGGCCTTTT TACGGTTCCT GGCCTTTTGC TGGCCTTTTG CTCACATGTT
7921 CTTTCCTGCG TTATCCCTTG ATTCTGTGGA TAACCGTATT ACCGCCTTTG AGTGAGCTGA
7981 TACCGCTCGC CGCAGCCGAA CGACCGAGCG CAGCGAGTCA GTGAGCGAGG AAGCGGAAGA
8041 GCGCCCAATA CGCAAACCGC CTCTCCCCGC GCGTTGGCCG ATTCATTAAAT GCAGCTGGCA
8101 CGACAGGTTT CCCGACTGGA AAGCGGGCAG TGAGCGCAAC GCAATTAATG TGAGTTACCT
8161 CACTCATTAG GCACCCAGG CTTTACACTT TATGCTTCCG GCTCCTATGT TGTGTGGAAT
8221 TGTGAGCGGA TAACAATTTT ACACAGGAAA CAGCTATGAC CATGATTACG CCAAGCTCGG
8281 AATTAACCTT CACTAAAGGG AACAAAAGCT GGGTACCGGG CCCCCCTCG AGATCCGGGA
8341 TCGAAGAAAT GATGGTAAAT GAAATAGGAA ATCAAGGAGC ATGAAGGCAA AAGACAAATA
8401 TAAGGGTCTGA ACGAAAAATA AAGTGAAAAG TGTTGATATG ATGTATTTGG CTTTGCGGCG
8461 CCGAAAAAAC GAGTTTACGC AATTGCACAA TCATGCTGAC TCTGTGGCGG ACCCGCGCTC
8521 TTGCCGGCCC GCGGATAACG CTGGGCGTGA GGCTGTGCCC GCGGAGTTT TTTGCGCCTG
8581 CATTITCCAA GGTITACCCT GCGCTAAGGG GCGAGATTGG AGAAGCAATA AGAATGCCGG
8641 TTGGGGTTGC GATGATGACG ACCACGACAA CTGGTGTCAT TATTTAAGTT GCCGAAAGAA
8701 CCTGAGTGCA TTTGCAACAT GAGTATACTA GAAGAATGAG CCAAGACTTG CGAGACGCGA
8761 GTTTGCCGGT GGTGCGAACA ATAGAGCGAC CATGACCTTG AAGGTGAGAC GCGCATAACC
8821 GCTAGAGTAC TTTGAAGAGG AAACAGCAAT AGGGTTGCTA CCAGTATAAA TAGACAGGTA
8881 CATAACAAC TGGAAATGGT TGTCTGTTTG AGTACGCTTT CAA

Figure 420

120/240

pDEST23

His6 carboxy-fusion vector, T7 promoter,

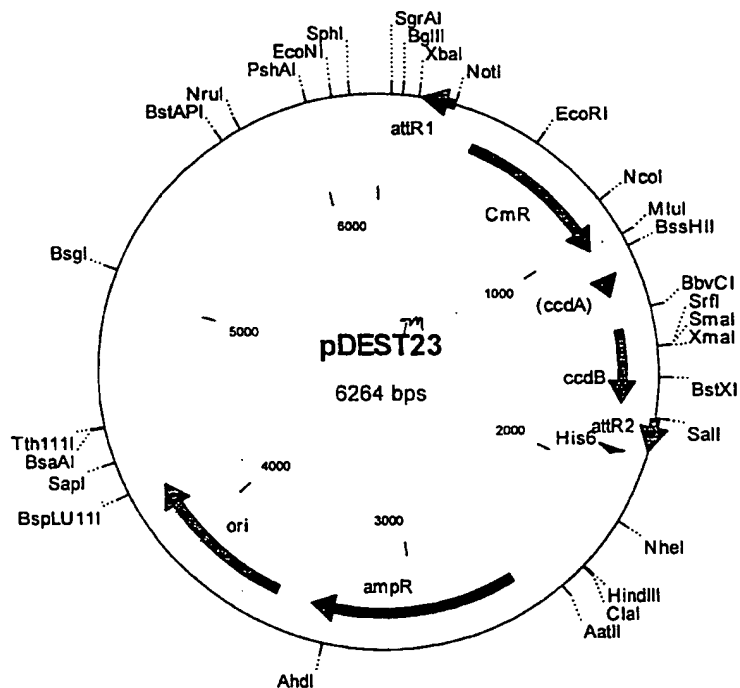
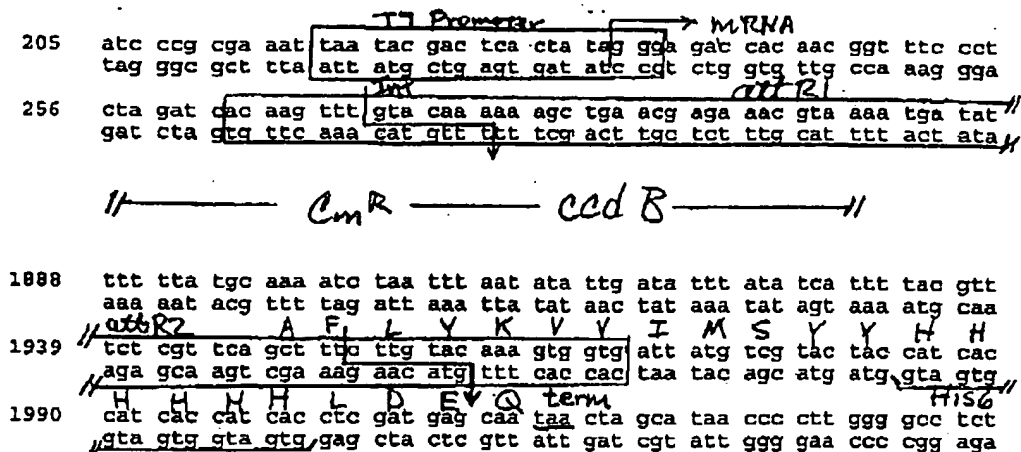


FIGURE 43A

pDEST23 6264 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
285..161		attR1
394..1053		CmR
1173..1257		inactivated ccdA
1395..1700		ccdB
1741..1865		attR2
1883..1911		his6
2574..3434		ampR
3583..4222		ori

1	TCTTCCCAT	CGGTGATGTC	GGCGATATAG	GCGCCAGCAA	CCGCACCTGT	GGCGCCGGTG
61	ATGCCGGCCA	CGATGCGTCC	GGCGTAGAGG	ATCGAGATCT	CGATCCCGCG	AAATTAATAC
121	GACTCACTAT	AGGGAGACCA	CAACGGTTTC	CCTCTAGATC	ACAAGTTTGT	ACAAAAAAGC
181	TGAACGAGAA	ACGTAAAATG	ATATAAATAT	CAATATATTA	AATTAGATTT	TGCATAAAAA
241	ACAGACTACA	TAATACTGTA	AAACACAACA	TATCCAGTCA	CTATGGCGGC	CGCATTAGGC
301	ACCCAGGCT	TTACACTTTA	TGCTTCCGGC	TCGTATAATG	TGTGGATTTT	GAGTTAGGAT
361	CCGGCGAGAT	TTTCAGGAGT	TAAGGAAGCT	AAAATGGAGA	AAAAAATCAC	TGGATATACC
421	ACCGTTGATA	TATCCCAATG	GCATCGTAAA	GAACATTTTG	AGGCATTTCA	GTCAGTTGCT
481	CAATGTACCT	ATAACCAGAC	CGTTCAGCTG	GATATTACGG	CCTTTTTTAA	GACCGTAAAG
541	AAAAATAAGC	ACAAGTTTTA	TCCGGCCTTT	ATTCACATTC	TGCCCCGCCT	GATGAATGCT
601	CATCCGGAAT	TCCGTATGCG	AATGAAAAGAC	GGTGAGCTGG	TGATATGGGA	TAGTGTTCAC
661	CCTTGTTACA	CCGTTTTCCA	TGAGCAAAC	GAAACGTTTT	CATCGCTCTG	GAGTGAATAC
721	CACGACGATT	TCCGGCAGTT	TCTACACATA	TATTCGCAAG	ATGTGGCGTG	TTACGGTGAA
781	AACCTGGCCT	ATTTCCCTAA	AGGGTTTATT	GAGAATATGT	TTTTCGTCTC	AGCCAATCCC
841	TGGGTGAGTT	TCACCAAGTT	TGATTTAAAC	GTGGCCAATA	TGGACAAC	CTTCGCCCCC
901	GTTTTACCA	TGGGCAAATA	TTATACGCAA	GGCGACAAGG	TGCTGATGCC	GCTGGCGATT
961	CAGGTTTCATC	ATGCCGCTCG	TGATGGCTTC	CATGTCGGCA	GAATGCTTAA	TGAATTACAA
1021	CAGTACTGCG	ATGAGTGGCA	GGGCGGGGCG	TAAACGCGTG	GATCCGGCTT	ACTAAAAGCC
1081	AGATAACAGT	ATGCGTATTT	GCGCCTGAT	TTTGCGGTA	TAAGAATATA	TACTGATATG
1141	TATACCCGAA	GTATGTCAAA	AAGAGGTGTG	CTATGAAGCA	GCGTATTACA	GTGACAGTTG
1201	ACAGCGACAG	CTATCAGTTG	CTCAAGGCAT	ATATGATGTC	AATATCTCCG	GTCTGGTAAG
1261	CACAACCATG	CAGAATGAAG	CCCGTCGTCT	GCGTGCCGAA	CGCTGGAAAG	CGGAAAATCA
1321	GGAAGGGATG	GCTGAGGTG	CCCGGTTTAT	TGAAATGAAC	GGCTCTTTTG	CTGACGAGAA
1381	CAGGGACTGG	TGAAATGCAG	TTTAAGGTTT	ACACCTATAA	AAGAGAGAGC	CGTTATCGTC
1441	TGTTTGTGGA	TGTACAGAGT	GATATTATTG	ACACGCCCGG	GCGACGGATG	GTGATCCCCC
1501	TGGCCAGTGC	ACGTCTGCTG	TCAGATAAAG	TCTCCCGTGA	ACTTTACCCG	GTGGTGCATA
1561	TCGGGGATGA	AAGCTGGCGC	ATGATGACCA	CCGATATGGC	CAGTGTGCCG	GTCTCCGTTA
1621	TCGGGGGAAGA	AGTGGCTGAT	CTCAGCCACC	GCGAAAATGA	CATCAAAAAC	GCCATTAAAC
1681	TGATGTTCTG	GGGAATATAA	ATGTCAGGCT	CCCTTATACA	CAGCCAGTCT	GCAGGTCGAC
1741	CATAGTGACT	GGATATGTTG	TGTTTACAG	TATTATGTAG	TCTGTTTTTT	ATGCAAAATC
1801	TAATTTAATA	TATTGATATT	TATATCATTT	TACGTTTCTC	GTTTACGCTT	CTTGTAACAA
1861	GTGGTGATTA	TGTCGTACTA	CCATCACCAT	CACCATCACC	TCGATGAGCA	ATAACTAGCA
1921	TAACCCCTTG	GGGCCTCTAA	ACGGGTCTTG	AGGGGTTTTT	TGCTGAAAGG	AGGAACTATA
1981	TCCGGATATC	CACAGGACGG	GTGTGGTCGC	CATGATCGCG	TAGTCGATAG	TGGCTCCAAG
2041	TAGCGAAGCG	AGCAGGACTG	GGCGGCGGCC	AAAGCGGTCG	GACAGTGCTC	CGAGAACGGG
2101	TGCGCATAGA	AATTGCATCA	ACGCATATAG	CGCTAGCAGC	ACGCCATAGT	GACTGGCGAT
2161	GCTGTGCGAA	TGGACGATAT	CCCGCAAGAG	GCCCGGCAGT	ACCGGCATAA	CCAAGCCTAT
2221	GCCTACAGCA	TCCAGGGTGA	CGGTGCCGAG	GATGACGATG	AGCGCATTGT	TAGATTTTAT
2281	ACACGGTGCC	TGACTGCGTT	AGCAATTTAA	CTGTGATAAA	CTACCGCATT	AAAGCTTATC
2341	GATGATAAGC	TGTCAAACAT	GAGAATTCTT	GAAGAAGAAA	GGGCCTCGTG	ATACGCCTAT
2401	TTTTATAGGT	TAATGTCTAG	ATAATAATGG	TTTCTTAGAC	GTCAGGTGGC	ACTTTTCGGG
2461	GAAATGTGCG	CGGAACCCCT	ATTTGTTTAT	TTTCTAAAT	ACATTCAAAT	ATGTATCCGC
2521	TCATGAGACA	ATAACCCTGA	TAAATGCTTC	AATAATATTG	AAAAAGGAAG	AGTATGAGTA
2581	TTCAACATTT	CCGTGTCGCC	CTTATTCCCT	TTTTTGCGGC	ATTTTGCCCT	CCTGTTTTTG
2641	CTACCCAGA	AACGCTGGTG	AAAGTAAAAG	ATGCTGAAGA	TCAGTTGGGT	GCACGAGTGG-

FIGURE 43B

122/240

```

2701 GTTACATCGA ACTGGATCTC AACAGCGGTA AGATCCTTGA GAGTTTTTCGC CCCGAAGAAG
2761 GTTTTCCAAT GATGAGCACT TTTAAAGTTC TGCTATGTGG CGCGGTATTA TCCCGTGTGG
2821 ACGCCGGGCA AGAGCAACTC GGTGCGCCGA TACACTATTC TCAGAATGAC TTGGTTGAGT
2881 ACTCACCAGT CACAGAAAAG CATCTTACGG ATGGCATGAC AGTAAGAGAA TTATGCAGTG
2941 CTGCCATAAC CATGAGTGAT AACACTGCGG CCAACTTACT TCTGACAACG ATCGGAGGAC
3001 CGAAGGAGCT AACCGCTTTT TTGCACAACA TGGGGGATCA TGTAACTCGC CTTGATCGTT
3061 GGGAAACCGGA GCTGAATGAA GCCATACCAA ACGACGAGCG TGACACCACG ATGCCTGCAG
3121 CAATGGCAAC AACGTTGCGC AAATAATTAA CTGGCGAACT ACTTACTCTA GCTTCCCGGC
3181 AACAAATTAAT AGACTGGATG GAGGCGGATA AAGTTGCAGG ACCACTTCTG CGCTCGGCCC
3241 TTCCGGCTGG CTGGTTTATT GCTGATAAAT CTGGAGCCGG TGAGCGTGGG TCTCGCGGTA
3301 TCATTGCAGC ACTGGGGCCA GATGGTAAGC CCTCCCGTAT CGTAGTTATC TACACGACGG
3361 GGAGTCAGGC AACTATGGAT GAACGAAATA GACAGATCGC TGAGATAGGT GCCTCACTGA
3421 TTAAGCATTG GTAAGTGTCA GACCAAGTTT ACTCATATAT ACTTTAGATT GATTTAAAGC
3481 TTCATTTTAA ATTTAAAAGG ATCTAGGTGA AGATCCTTTT TGATAATCTC ATGACCAAAA
3541 TCCCTTAAAG TGAGTTTTTCG TTCCACTGAG CGTCAGACCC CGTAGAAAAG ATCAAAGGAT
3601 CTCTTTGAGA TCCTTTTTTT CTGCGCGTAA TCTGCTGCTT GCAAACAAA AAACCACCGC
3661 TACCAGCGGT GGTGTTGTTG CCGGATCAAG AGCTACCAAC TCTTTTTCCG AAGGTAAGTG
3721 GCTTCAGCAG AGCGCAGATA CCAAATACGT TCCTTCTAGT GTAGCCGTAG TTAGGCCACC
3781 ACTTCAAGAA CTCTGTAGCA CCGCTACAT ACCTCGCTCT GCTAATCCTG TTACAGTGG
3841 CTGCTGCCAG TGGCGATAAG TCGTGTCTTA CCGGGTTGGA CTCAAGACGA TAGTTACCGG
3901 ATAAGGCGCA GCGGTCGGGC TGAACGGGGG GTTCGTGCAC ACAGCCCAGC TTGGAGCGAA
3961 CGACCTACAC CGAAGTGAGA TACCTACAGC GTGAGCTATG AGAAAGCGCC ACGCTTCCCG
4021 AAGGGAGAAA GCGGACAGG TATCCGGTAA GCGGAGGGT CGGAACAGGA GAGCGCACGA
4081 GGGAGCTTCC AGGGGAAAC GCCTGGTATC TTTATAGTCC TGTCGGGTTT CGCCACCTCT
4141 GACTTGAGCG TCGATTTTGG TGATGCTCGT CAGGGGGGCG GAGCCTATGG AAAACGCCA
4201 GCAACGCGGC CTTTTTACGG TTCTGCGCT TTTGCTGGCC TTTTGCTCAC ATGTTCTTTC
4261 CTGCGTTATC CCCTGATTCT GTGGATAACC GTATTACCGC CTTTGAGTGA GCTGATACCG
4321 CTCGCCGAG CCGAACGACC GAGCGCAGCG AGTCAGTGAG CGAGGAAGCG GAAGAGCGCC
4381 TGATGCGGTA TTTTCTCCTT ACGCATCTGT GCGGTATTTT ACACCGCATA TATGGTGCAC
4441 TCTCAGTACA ATCTGCTCTG ATGCCGCATA GTTAAGCCAG TATACACTCC GCTATCGCTA
4501 CGTGACTGGG TCATGGCTGC GCGCCGACAC CCGCCAACAC CCGCTGACGC GCCCTGACGG
4561 GCTTGTCTGC TCCCGGCATC CGCTTACAGA CAAGCTGTGA CCGTCTCCGG GAGCTGCATG
4621 TGTCAGAGGT TTTTACCCTC ATCACCAGAA CGCGCGAGGC AGCTGCGGTA AAGCTCATCA
4681 GCGTGGTCTG GAAGCGATTG ACAGATGTCT GCCTGTTTCT CCGCTCCAG CTCTGAGT
4741 TTCTCCAGAA GCGTTAATGT CTGGCTTCTG ATAAAGCGGG CCATGTTAAG GCGGTTTTTT
4801 TCCTGTTTGG TCACTGATGC CTCCGTGTAA GGGGGATTTC TGTTTCATGGG GGTAATGATA
4861 CCGATGAAAC GAGAGAGGAT GCTCAGGATA CCGGTTACTG ATGATGAACA TGCCCGGTTA
4921 CTGGAACGTT GTGAGGGTAA ACAACTGGCG GTATGGATGC GCGGGGACCA GAGAAAAATC
4981 ACTCAGGGTC AATGCCAGCG CTTCGTTAAT ACAGATGTAG GTGTTCCACA GGGTAGCCAG
5041 CAGCATCCTG CGATGCAGAT CCGGAACATA ATGGTGCAGG GCGCTGACTT CCGCGTTTCC
5101 AGACTTTACG AAACACGGAA ACCGAAGACC ATTCATGTTG TTGCTCAGGT CGCAGACGTT
5161 TTGCAGCAGC AGTCGCTTCA CGTTCGCTCG CGTATCGGTG ATTCAATTCT CTAACCAGTA
5221 AGGCAACCCC GCCAGCCTAG CCGGCTCTC AACGACAGGA GCACGATCAT GCGCACCCGT
5281 GGCCAGGACC CAACGCTGCC CGAGATGCGC CGCGTGGGAT TGCTGGAGAT GCGGACGCG
5341 ATGGATATGT TCTGCCAAGG GTTGGTTTGC GCATTACAG TTCTCCGCAA GAATTGATTG
5401 GCTCCAATTC TTGGAGTGGT GAATCCGTTA GCGAGGTGCC GCCGGCTTCC ATTCAAGTCC
5461 AGGTGGCCCG GCTCCATGCA CCGCGACGCA ACGCGGGGAG GCAGACAAGG TATAGGGCGG
5521 CGCCTACAAT CCATGCCAAC CCGTTCCATG TGCTCGCCGA GCGGACATA ATCGCCGTGA
5581 CGATCAGCGG TCCAGTGATC GAAGTTAGGC TGGTAAGAGC CGCGAGCGAT CCTTGAAGCT
5641 GTCCCTGATG GTCGTCATCT ACCTGCCTGG ACAGCATGGC CTGCAACGCG GGCATCCCGA
5701 TGCCGCCGGA AGCGAGAAGA ATCATAATGG GGAAGGCCAT CCAGCCTCGC GTCGCGAACG
5761 CCAGCAAGAC GTAGCCAGC GCGTCGGCCG CCATGCCGGC GATAATGGCC TGCTTCTCGC
5821 CGAAACGTTT GGTGCGGGA CCAAGTGACG AGGCTTGAGC GAGGGCGTGC AAGATTCCGA
5881 ATACCGCAAG CGACAGGCCG ATCATCGTCG CGCTCCAGCG AAAGCGGTCC TCGCCGAAAA
5941 TGACCCAGAG CGCTGCCGGC ACCTGTCCTA CGAGTTGCAT GATAAAGAAG ACAGTCATAA
6001 GTGCGGCGAC GATAGTCATG CCCCGGCCCC ACCGGAAGGA GCTGACTGGG TTGAAGGCTC
6061 TCAAGGGCAT CGGTGATCG ACGCTCTCCC TTATGCGACT CCTGCATTAG GAAGCAGCCC
6121 AGTAGTAGGT TGAGGCCGTT GAGCACCGCC GCCGCAAGGA ATGGTGCATG CAAGGAGATG-

```

Figure 43C

123/240

6181 GCGCCCAACA GTCCCCCGGC CACGGGGCCT GCCACCATAC CCACGCCGAA ACAAGCGCTC
6241 ATGAGCCCGA AGTGGCGAGC CCGA

FIGURE 43D

125/240

pDEST24 6961 bp

<u>Location (Base Nos.)</u>	<u>Gene Encoded</u>
195..71	attR1
304..963	CmR
1083..1167	inactivated ccdA
1305..1610	ccdB
1651..1775	attR2
1783..2451	GST
3181..4041	ampR
4190..4829	ori

```

1 ATCGAGATCT CGATCCCGCG AAATTAATAC GACTCACTAT AGGGAGACCA CAACGGTTTC
61 CCTCTAGATC ACAAGTTTGT AAAAAAAGC TGAACGAGAA ACGTAAATG ATATAAATAT
121 CAATATATTA AATTAGATTT TGCATAAAAA ACAGACTACA TAATACTGTA AAACACAACA
181 TATCCAGTCA CTATGGCGGC CGCATTAGGC ACCCCAGGCT TTACACTTTA TGCTTCCGGC
241 TCGTATAATG TGTGGATTTT GAGTTAGGAT CCGGCGAGAT TTTCAGGAGC TAAGGAAGCT
301 AAAATGGAGA AAAAAATCAC TGGATATACC ACCGTTGATA TATCCCAATG GCATCGTAAA
361 GAACATTTTG AGGCATTTC GTCAGTTGCT CAATGTACCT ATAACCAGAC CGTTCAGCTG
421 GATATTACGG CCTTTTAAAA GACCGTAAAG AAAAATAAGC ACAAGTTTTA TCCGGCCTTT
481 ATTCACATTC TTGCCCCTCT GATGAATGCT CATCCGGAAT TCCGTATGGC AATGAAAGAC
541 GGTGAGCTGG TGATATGGGA TAGTGTTCAC CCTTGTTACA CCGTTTTTCCA TGAGCAAAC
601 GAAACGTTTT CATCGCTCTG GAGTGAATAC CACGACGATT TCCGGCAGTT TCTACACATA
661 TATTCGCAAG ATGTGGCGTG TTACGGTGAA AACCTGGCCT ATTTCCCTAA AGGGTTTATT
721 GAGAATATGT TTTTCGTCTC AGCCAATCCC TGGGTGAGTT TCACCAGTTT TGATTTAAAC
781 GTGGCCAATA TGGACAACCT CTTCGCCCCC GTTTTCACCA TGGGCAAATA TTATACGCAA
841 GGCGACAAGG TGCTGATGCC GCTGGCGATT CAGGTTTCATC ATGCCGCTCTG TGATGGCTTC
901 CATGTCGGCA GAATGCTTAA TGAATTACAA CAGTACTGCG ATGAGTGGCA GGGCGGGGCG
961 TAAACGCGTG GATCCGGCTT ACTAAAAGCC AGATAACAGT ATGCGTATTT GCGCGCTGAT
1021 TTTTGGCGTA TAAGAATATA TACTGATATG TATACCCGAA GTATGTCAAA AAGAGGTGTG
1081 CTATGAAGCA GCGTATTACA GTGACAGTTG ACAGCGACAG CTATCAGTTG CTCAAGGCAT
1141 ATATGATGTC AATATCTCCG GTCTGGTAAG CACAACCATG CAGAATGAAG CCCGTCGTCT
1201 GCGTGCCGAA CGCTGGAAAG CGGAAAATCA GGAAGGGATG GCTGAGGTCG CCCGGTTTAT
1261 TGAAATGAAC GGCTCTTTTG GTGACGAGAA CAGGGACTGG TGAATGCAG TTTAAGGTTT
1321 ACACCTATAA AAGAGAGAGC CGTTATCGTC TGTTTGTGGA TGACAGAGT GATATTATTG
1381 ACACGCCCCG GCGACGGATG GTGATCCCCC TGGCCAGTGC ACGTCTGCTG TCAGATAAAG
1441 TCTCCCGTGA ACTTTACCCG GTGGTGCATA TCGGGGATGA AAGCTGGCGC ATGATGACCA
1501 CCGATATGGC CAGTGTGCCG GTCTCCGTTA TCGGGGAAGA AGTGGCTGAT CTCAGCCACC
1561 GCGAAAATGA CATCAAAAAC GCCATTAAAC TGATGTTCTG GGGAATATAA ATGTCAGGCT
1621 CCCTTATACA CAGCCAGTCT GCAGGTCGAC CATAGTGAAT GGATATGTTG TGTTTTACAG
1681 TATTATGTAG TCTGTTTTTT ATGCAAAATC TAATTTAATA TATTGATATT TATATCATT
1741 TACGTTTCTC GTTCAGCTTT CTTGTACAAA GTGGTGATTA TGTCCCTAT ACTAGTTTAT
1801 TGGAAAATTA AGGGCCTTGT GCAACCCACT CGACTTCTTT TGAATATCT TGAAGAAAAA
1861 TATGAAGAGC ATTTGTATGA GCGCGATGAA GGTGATAAAT GGCGAAACAA AAAGTTTGAA
1921 TTGGGTTTGG AGTTTCCCAA TCTTCCTTAT TATATTGATG GTGATGTTAA ATTAACACAG
1981 TCTATGGCCA TCATACGTTA TATAGCTGAC AAGCACAACA TGTGGGTGG TTGTCCAAAA
2041 GAGCGTGCAG AGATTTCAAT GCTTGAAGGA GCGGTTTTGG ATATTAGATA CGGTGTTTTG
2101 AGAATTGCAT ATAGTAAAGA CTTTGAAACT CTCAAAGTTG ATTTTCTTAG CAAGCTACCT
2161 GAAATGCTGA AAATGTTTCA AGATCGTTTA TGTCAAAAAA CATATTTAAA TGGTGATCAT
2221 GTAACCCATC CTGACTTCAT GTTGATGAC GCTCTTGATG TTGTTTATA CATGGACCCA
2281 ATGTGCCCTG ATGCGTTCCC AAAATTAGTT TGTTTTAAAA AACGTATTGA AGCTATCCCA
2341 CAAATTGATA AGTACTTGAA ATCCAGCAAG TATATAGCAT GGCTTTGCA GGGCTGGCAA
2401 GCCACGTTTG GTGGTGGCGA CCATCCTCCA AAATCGGATC TGGTTCCGCG TCCATGGGGA
2461 TCCGGCTGCT AACAAAGCCC GAAAGGAAGC TGAGTTGGCT GCTGCCACCG CTGAGCAATA
2521 ACTAGCATAA CCCCTTGGGG CCTCTAAACG GGTCTTGAGG GGTTTTTTGC TGAAAGGAGG
2581 AACTATATCC GGATATCCAC AGGACGGGTG TGGTCGCCAT GATCGCGTAG TCGATAGTGG
2641 CTCCAAGTAG CGAAGCGAGC AGGACTGGGC GGCGGCCAAA GCGGTCCGAC AGTGCTCCGA-

```

FIGURE 44B

126/240

2701 GAACGGGTGC GCATAGAAAT TGCATCAACG CATATAGCGC TAGCAGCACG CCATAGTGAC
2761 TGGCGATGCT GTCGGAATGG ACGATATCCC GCAAGAGGCC CGGCAGTACC GGCATAACCA
2821 AGCCTATGCC TACAGCATCC AGGGTGACGG TGCCGAGGAT GACGATGAGC GCATTGTTAG
2881 ATTCATACA CGGTGCCTGA CTGCGTTAGC AATTAACTG TGATAAACTA CCGCATTAA
2941 GCTTATCGAT GATAAGCTGT CAAACATGAG AATTCTTGAA GACGAAAGGG CCTCGTGATA
3001 CGCCTATTTT TATAGGTTAA TGTCATGATA ATAATGGTTT CTTAGACGTC AGGTGGCACT
3061 TTTCGGGGAA ATGTGCGCGG AACCCCTATT TGTTTATTTT TCTAAATACA TTCAAATATG
3121 TATCCGCTCA TGAGACAATA ACCCTGATAA ATGCTTCAAT AATATTGAAA AAGGAAGAGT
3181 ATGAGTATTC AACATTTCCG TGTGCGCCTT ATCCCTTTT TTGCGGCATT TTGCCTTCCT
3241 GTTTTGTCT ACCCAGAAAC GCTGGTGAAA GTAAAAGATG CTGAAGATCA GTTGGGTGCA
3301 CGAGTGGGTT ACATCGAACT GGATCTCAAC AGCGGTAAGA TCCTTGAGAG TTTTCGCCCC
3361 GAAGAACGTT TTCCAATGAT GAGCACTTTT AAAGTTCTGC TATGTGGCGC GGTATTATCC
3421 CGTGTGACG CCGGGCAAGA GCAACTCGGT CGCCGCATAC ACTATTCTCA GAATGACTTG
3481 GTTGAGTACT CACCAGTCAC AGAAAAGCAT CTTACGGATG GCATGACAGT AAGAGAATTA
3541 TGCAGTGCTG CCATAACCAT GAGTGATAAC ACTGCGGCCA ACTTACTTCT GACAACGATC
3601 GGAGGACCGA AGGAGCTAAC CGCTTTTTTG CACAACATGG GGGATCATGT AACTCGCCTT
3661 GATCGTTGGG AACCGGAGCT GAATGAAGCC ATACCAAACG ACGAGCGTGA CACCACGATG
3721 CCTGCAGCAA TGGCAACAAC GTTGCGCAA CTATTAACTG GCGAACTACT TACTCTAGCT
3781 TCCCGGCAAC AATTAATAGA CTGGATGGAG GCGGATAAAG TTGCAGGACC ACTTCTGCGC
3841 TCGGCCCTTC CGGCTGGCTG GTTTATTGCT GATAAATCTG GAGCCGGTGA GCGTGGGTCT
3901 CGCGGTATCA TTGCAGCACT GGGGCCAGAT GGTAAGCCCT CCCGTATCGT AGTTATCTAC
3961 ACGACGGGGA GTCAGGCAAC TATGGATGAA CGAAATAGAC AGATCGCTGA GATAGGTGCC
4021 TCACTGATTA AGCATTGGTA ACTGTCAGAC CAAGTTTACT CATATATACT TTAGATTGAT
4081 TTAAAACCTT ATTTTAAATT TAAAAGGATC TAGGTGAAGA TCCTTTTTGA TAATCTCATG
4141 ACCAAAATCC CTTAACGTGA GTTTTCGTTT CACTGAGCGT CAGACCCCGT AGAAAAGATC
4201 AAAGGATCTT CTTGAGATCC TTTTTTCTG CGCGTAATCT GCTGCTTGCA AACAAAAA
4261 CCACCGCTAC CAGCGGTGGT TTGTTTGCGG GATCAAGAGC TACCAACTCT TTTTCCGAAG
4321 GTAACCTGGT TCAGCAGAGC GCAGATACCA AATACTGTCC TTCTAGTGTA GCCGTAGTTA
4381 GGCCACCACT TCAAGAACTC TGTAGCACCG CCTACATACC TCGCTCTGCT AATCCTGTTA
4441 CCAGTGGCTG CTGCCAGTGG CGATAAGTCG TGTCTTACCG GGTGGAATC AAGACGATAG
4501 TTACCGGATA AGGCGCAGCG GTCGGGCTGA ACGGGGGGTT CGTGACACA GCCCAGCTTG
4561 GAGCGAACGA CCTACACCGA ACTGAGATAC CTACAGCGTG AGCTATGAGA AAGCGCCACG
4621 CTTCCCGAAG GGAGAAAGGC GGACAGGTAT CCGGTAAGCG GCAGGGTCGG AACAGGAGAG
4681 CGCACGAGGG AGCTTCCAGG GGGAAACGCC TGGTATCTTT ATAGTCCTGT CGGGTTTCGC
4741 CACCTCTGAC TTGAGCGTCG ATTTTGTGA TGCTCGTCAG GGGGGCGGAG CCTATGGAAA
4801 AACGCCAGCA ACGCGGCCTT TTACCGTTTC CTGGCCTTTT GCTGGCCTTT TGCTCACATG
4861 TTCTTCTCTG CGTTATCCCC TGATTCTGTG GATAACCGTA TTACCGCCTT TGAGTGAGCT
4921 GATACCGCTC GCCGCAGCCG AACGACCGAG CGCAGCGAGT CAGTGAGCGA GGAAGCGGAA
4981 GAGCGCCTGA TGCGGTATTT TCTCCTTACG CATCTGTGCG GTATTTTACA CCGCATATAT
5041 GGTGCACTCT CAGTACAATC TGCTCTGATG CCGCATAGTT AAGCCAGTAT ACACTCCGCT
5101 ATCGCTACGT GACTGGGTCA TGGCTGCGCC CCGACACCCG CCAACACCCG CTGACGCGCC
5161 CTGACGGGCT TGTCTGCTCC CGGCATCCGC TTACAGACAA GCTGTGACCG TCTCCGGGAG
5221 CTGCATGTGT CAGAGGTTTT CACCGTCATC ACCGAAACGC GCGAGGCAGC TGGCGTAAAG
5281 CTCATCAGCG TGGTCGTGAA GCGATTACCA GATGTCTGCC TGTTCATCCG CGTCCAGCTC
5341 GTTGAGTTTC TCCAGAAGCG TTAATGTCTG GCTTCTGATA AAGCGGGCCA TGTTAAGGGC
5401 GGTTTTTTCC TGTTTGGTCA CTGATGCCTC CGTGTAAAGG GGATTTCTGT TCATGGGGGT
5461 AATGATACCG ATGAAACGAG AGAGGATGCT CACGATACGG GTTACTGATG ATGAACATGC
5521 CCGGTTACTG GAACGTTGTG AGGTTAAACA ACTGGCGGTA TGGATGCGGC GGGACCAGAG
5581 AAAAATCACT CAGGGTCAAT GCCAGCGCTT CGTTAATACA GATGTAGGTG TTCCACAGGG
5641 TAGCCAGCAG CATCTGCGA TGCGATCCG GAACATAATG GTGCAGGGCG CTGACTTCCG
5701 CGTTTCCAGA CTTTACGAAA CACGGAACCC GAAGACCATT CATGTTGTTG CTCAGGTCGC
5761 AGACGTTTTG CAGCAGCAGT CGCTTACGCT TCGCTCGCGT ATCGGTGATT CATCTGCTA
5821 ACCAGTAAGG CAACCCCGCC AGCCTAGCCG GGTCTCAAC GACAGGAGCA CGATCATGCG
5881 CACCCGTGGC CAGGACCCAA CGCTGCCCGA GATGCGCCGC GTGCGGCTGC TGGAGATGGC
5941 GGACGCGATG GATATGTTCT GCCAAGGGTT GGTGTGCGCA TTCACAGTTC TCCGCAAGAA
6001 TTGATTGGCT CCAATTCTTG GAGTGGTGAA TCCGTTAGCG AGGTGCCGCC GGCTTCCATT
6061 CAGGTCGAGG TGGCCCGGCT CCATGCACCG CGACGCAACG CGGGGAGGCA GACAAGGTAT
6121 AGGGCGGCGC CTACAATCCA TGCCAACCCG TTCCATGTGC TCGCCGAGGC GGCATAAATC

Figure 44C

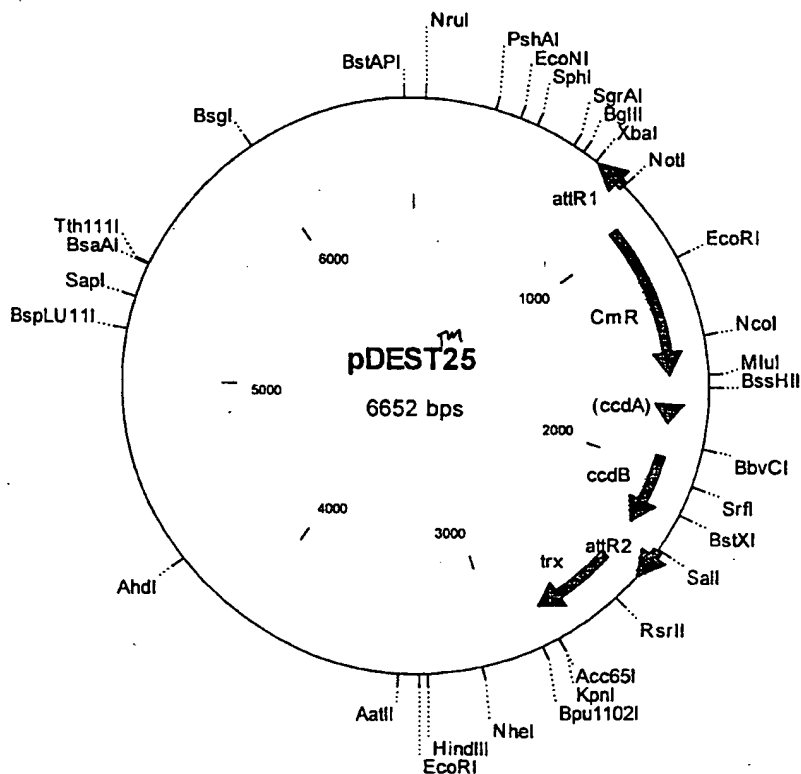
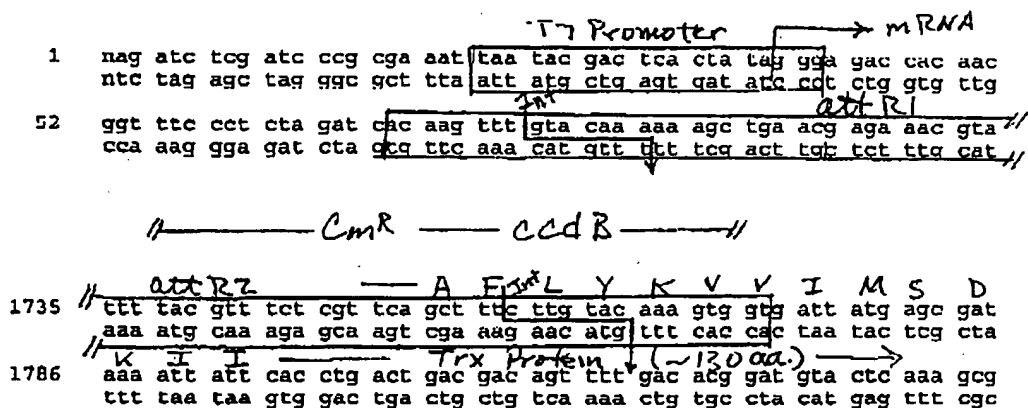
127/240

6181 GCCGTGACGA TCAGCGGTCC AGTGATCGAA GTTAGGCTGG TAAGAGCCGC GAGCGATCCT
6241 TGAAGCTGTC CCTGATGGTC GTCATCTACC TGCCTGGACA GCATGGCCTG CAACGCGGGC
6301 ATCCCGATGC CGCCGGAAGC GAGAAGAATC ATAATGGGGA AGGCCATCCA GCCTCGCGTC
6361 GCGAACGCCA GCAAGACGTA GCCCAGCGCG TCGGCCGCCA TGCCGGCGAT AATGGCCTGC
6421 TTCTCGCCGA AACGTTTGGT GCGGGGACCA GTGACGAAGG CTTGAGCGAG GCGGTGCAAG
6481 ATTCCGAATA CCGCAAGCGA CAGGCCGATC ATCGTCGCGC TCCAGCGAAA GCGGTCTCTG
6541 CCGAAAATGA CCCAGAGCGC TGCCGGCACC TGTCCTACGA GTTGCATGAT AAAGAAGACA
6601 GTCATAAGTG CGGCGACGAT AGTCATGCCC CGCGCCCACC GGAAGGAGCT GACTGGGTTG
6661 AAGGCTCTCA AGGGCATCGG TCGATCGACG CTCTCCCTTA TCGGACTCCT GCATTAGGAA
6721 GCAGCCCACT AGTAGGTTGA GGCCGTTGAG CACCGCCGCC GCAAGGAATG GTGCATGCAA
6781 GGAGATGGCG CCCAACAGTC CCCCAGCCAC GGGGCTGCC ACCATACCCA CGCCGAAACA
6841 AGCGCTCATG AGCCCGAAGT GGCGAGCCCG ATCTTCCCCA TCGGTGATGT CGGCGATATA
6901 GCGGCCAGCA ACCGCACCTG TGGCGCCGCT GATGCCGCC ACGATGCGTC CGGCGTAGAG
6961 G

FIGURE 44D

128/240
FIGURE 45A

pDEST25
Thioredoxin carboxy-fusion vector, T7 promoter



129/240

pDEST25 6652 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
844..720		attR1
953..1612		CmR
1732..1816		inactivated ccdA
1954..2259		ccdB
2300..2424		attR2
2432..2794		trx
1	CCGGAAGCGA GAAGAATCAT AATGGGGAAG GCCATCCAGC CTCGCGTCGC GAACGCCAGC	
61	AAGACGTAGC CCAGCGCGTC GGCCGCCATG CCGGCGATAA TGGCCTGCTT CTCGCCGAAA	
121	CGTTTGGTGG CGGGACCACT GACGAAGGCT TGAGCGAGGG CGTGCAGAT TCCGAATACC	
181	GCAAGCGACA GGCCGATCAT CGTCGCGCTC CAGCGAAAGC GGTCTCGCC GAAAATGACC	
241	CAGAGCGCTG CCGGCACCTG TCCTACGAGT TGCATGATAA AGAAGACAGT CATAAGTGCG	
301	GCGACGATAG TCATGCCCCG CGCCACCCGG AAGGAGCTGA CTGGGTTGAA GGCTCTCAAG	
361	GGCATCGGTC GATCGACGCT CTCCCTTATG CGACTCCTGC ATTAGGAAGC AGCCAGTAG	
421	TAGGTTGAGG CCGTTGAGCA CCGCCGCCGC AAGGAATGGT GCATGCAAGG AGATGGCGCC	
481	CAACAGTCCC CCGGCCACGG GGCTTGCCAC CATACCCACG CCGAAACAAG CGCTCATGAG	
541	CCCGAAGTGG CGAGCCCAGT CTTCCCATC GGTGATGTCG GCGATATAGG CGCCAGCAAC	
601	CGCACCTGTG GCGCCGGTGA TGCCGCCAC GATGCGTCCG GCGTAGAGGA TCGAGATCTC	
661	GATCCCGCGA AATTAATACG ACTCACTATA GGGAGACCAC AACGGTTTCC CTCTAGATCA	
721	CAAGTTTGTA CAAAAAGCT GAACGAGAAA CGTAAATGA TATAAATATC AATATATTAA	
781	ATTAGATTTT GCATAAAAA CAGACTACAT AATACTGTAA AACACAACAT ATCCAGTCAC	
841	TATGGCGGCC GCATTAGGCA CCCAGGCTT TACACTTTAT GCTTCCGGCT CGTATAATGT	
901	GTGGATTTTG AGTTAGGATC CGGCGAGATT TTCAGGAGCT AAGGAAGCTA AAATGGAGAA	
961	AAAAATCACT GGATATACCA CCGTTGATAT ATCCCAATGG CATCGTAAAG AACATTTTGA	
1021	GGCATTTTCAG TCAGTTGCTC AATGTACCTA TAACCAGACC GTTCAGCTGG ATATTACGGC	
1081	CTTTTAAAG ACCGTAAAGA AAAATAAGCA CAAGTTTAT CCGGCCTTTA TTCACATTCT	
1141	TGCCCCGCTG ATGAATGCTC ATCCGGAATT CCGTATGGCA ATGAAAGACG GTGAGCTGGT	
1201	GATATGGGAT AGTGTTCACC CTGTTACAC CGTTTTCCAT GAGCAAACG AAACGTTTTT	
1261	ATCGCTCTGG AGTGAATACC ACGACGATTT CCGGCAGTTT CTACACATAT ATTTCGAAGA	
1321	TGTGGCGTGT TACGGTGAAA ACCTGGCCTA TTTCCCTAAA GGGTTTATTG AGAATATGTT	
1381	TTTCGTCTCA GCCAATCCCT GGGTGAGTTT CACCAGTTT GATTTAAACG TGGCCAATAT	
1441	GGACAACTTC TTCGCCCCCG TTTCACCAT GGGCAAATAT TATACGCAAG GCGACAAGGT	
1501	GCTGATGCCG CTGGCGATTG AGGTTTCATCA TGCCGTCTGT GATGGCTTCC ATGTCGGCAG	
1561	AATGCTTAAT GAATTACAAC AGTACTGCGA TGAGTGGCAG GCGGGGCGT AAACGCGTGG	
1621	ATCCGGCTTA CTAAGGCCA GATAACAGTA TGCGTATTG CGCGCTGATT TTTGCGGTAT	
1681	AAGAATATAT ACTGATATGT ATACCCGAAG TATGTCAAAA AGAGGTGTGC TATGAAGCAG	
1741	CGTATTACAG TGACAGTTGA CAGCGACAGC TATCAGTTGC TCAAGGCATA TATGATGTCA	
1801	ATATCTCCGG TCTGGTAAGC ACAACCATGC AGAATGAAGC CCGTCGTCTG CGTGCCGAAC	
1861	GCTGGAAAGC GGAATATCAG GAAGGGATGG CTGAGGTCGC CCGGTTTATT GAAATGAACG	
1921	GCTCTTTTGC TGACGAGAAC AGGGACTGGT GAAATGCAGT TTAAGGTTTA CACCTATAAA	
1981	AGAGAGAGCC GTTATCGTCT GTTTGTGGAT GTACAGAGTG ATATTATTGA CAGCCCCGGG	
2041	CGACGGATGG TGATCCCCCT GGCCAGTGCA CGTCTGCTGT CAGATAAAGT CTCCCCTGAA	
2101	CTTTACCCGG TGGTGCATAT CCGGGATGAA AGCTGGCGCA TGATGACCAC CGATATGGCC	
2161	AGTGTGCCGG TCTCCGTTAT CCGGGGAAGAA GTGGCTGATC TCAGCCACCG CGAAAATGAC	
2221	ATCAAAAACG CCATTAACCT GATGTTCTGG GGAATATAAA TGTCAGGCTC CCTTATACAC	
2281	AGCCAGTCTG CAGGTCCAGC ATAGTGACTG GATATGTTGT GTTTTACAGT ATTATGTAGT	
2341	CTGTTTTTTA TGCAAAATCT AATTTAATAT ATTGATATT ATATCATTTT ACGTTTCTCG	
2401	TTCAGCTTTC TTGTACAAAG TGGTGATTAT GAGCGATAAA ATTATTACAC TGACTGACGA	
2461	CAGTTTTGAC ACGGATGTAC TCAAAGCGGA CCGGGCGATC CTCGTCGATT TCTGGGCAGA	
2521	GTGGTGCGGT CCGTGCAAAA TGATCGCCCC GATTCTGGAT GAAATCGCTG ACGAATATCA	
2581	GGGCAAACTG ACCGTTGCAA AACTGAACAT CGATCAAAAC CCTGGCACTG CGCCGAAATA	
2641	TGGCATCCGT GGTATCCCGA CTCTGCTGCT GTTCAAAAAC GGTGAAGTGG CGGCAACCAA	
2701	AGTGGGTGCA CTGTCTAAAG GTCAGTTGAA AGAGTTCCTC GACGCTAACC TGGCCGGTTC	
2761	TGGTCTGGT GATGACGATG ACAAGGTACC CCGGGATCGA TCCGGCTGCT AACAAAGCCC	

Figure 45B

130/240

2821 GAAAGGAAGC TGAGTTGGCT GCTGCCACCG CTGAGCAATA ACTAGCATAA CCCCTTGGGG
2881 CCTCTAAACG GGTCTTGAGG GGTTTTTTGC TGAAAGGAGG AACTATATCC GGATATCCAC
2941 AGGACGGGTG TGGTCGCCAT GATCGCGTAG TCGATAGTGG CTCCAAGTAG CGAAGCGAGC
3001 AGGACTGGGC GGCGGCCAAA GCGGTCCGAG AGTGCTCCGA GAACGGGTGC GCATAGAAAT
3061 TGCATCAACG CATATAGCGC TAGCAGCAGC CCATAGTGAC TGGCGATGCT GTCCGAATGG
3121 ACGATATCCC GCAAGAGGCC CGGCAGTACC GGCATAACCA AGCCTATGCC TACAGCATCC
3181 AGGGTGACGG TGCCGAGGAT GACGATGAGC GCATTGTTAG ATTTTCATACA CGGTGCCTGA
3241 CTGCGTTAGC AATTTAACTG TGATAAACTA CCGCATTAAA GCTTATCGAT GATAAGCTGT
3301 CAAACATGAG AATTCTTGAA GACGAAAGGG CCTCGTGATA CGCCTATTTT TATAGGTTAA
3361 TGTCATGATA ATAATGGTTT CTTAGACGTC AGGTGGCACT TTTCGGGGAA ATGTGCGCGG
3421 AACCCCTATT TGTTTTATTT TCTAAATACA TTCAAATATG TATCCGCTCA TGAGACAATA
3481 ACCCTGATAA ATGCTTCAAT AATATTGAAA AAGGAAGAGT ATGAGTATTC AACATTTCCG
3541 TGTCGCCCTT ATTCCCTTTT TTGCGGCATT TTGCCTTCCT GTTTTTGCTC ACCCAGAAAC
3601 GCTGGTGAAA GTAAAAGATG CTGAAGATCA GTTGGGTGCA CGAGTGGGT ACATCGAACT
3661 GGATCTCAAC AGCGGTAAGA TCCTTGAGAG TTTTCGCCCC GAAGAACGTT TTCCAATGAT
3721 GAGCACTTTT AAAGTTCTGC TATGTGGCGC GGTATTATCC CGTGTGACG CCGGGCAAGA
3781 GCAACTCGGT CGCCGCATAC ACTATTCTCA GAATGACTTG GTTGAGTACT CACCAGTCAC
3841 AGAAAAGCAT CTTACGGATG GCATGACAGT AAGAGAATTA TGCAGTGCTG CCATAACCAT
3901 GAGTGATAAC ACTGCGGCCA ACTTACTTCT GACAACGATC GGAGGACCGA AGGAGCTAAC
3961 CGCTTTTTTG CACAACATGG GGGATCATGT AACTCGCCTT GATCGTTGGG AACCGGAGCT
4021 GAATGAAGCC ATACCAAACG ACGAGCGTGA CACCACGATG CCTGCAGCAA TGGCAACAAC
4081 GTTGGCGAAA CTATTAAC TGCGAACTACT TACTCTAGCT TCCCGGCAAC AATTAATAGA
4141 CTGATGGAG GCGGATAAAG TTGCAGGACC ACTTCTGCGC TCGGCCCTTC CGGCTGGCTG
4201 GTTTATTGCT GATAAATCTG GAGCCGGTGA GCGTGGGTCT CGCGGTATCA TTGCAGCACT
4261 GGGGCCAGAT GGTAAGCCCT CCCGTATCGT AGTTATCTAC ACGACGGGGA GTCAGGCAAC
4321 TATGGATGAA CGAAATAGAC AGATCGTGTA GATAGGTGCC TCACTGATTA AGCATTGGTA
4381 ACTGTCAGAC CAAGTTTACT CATATATACT TTAGATTGAT TTAAAACTTC ATTTTAAAT
4441 TAAAGGATC TAGGTGAAGA TCCTTTTTGA TAATCTCATG ACCAAAATCC CTTAACGTGA
4501 GTTTTCGTTT CACTGAGCGT CAGACCCCGT AGAAAAGATC AAAGGATCTT CTTGAGTCC
4561 TTTTTTCTG CGCGTAATCT GCTGCTTGCA AACAAAAAAA CCACCGCTAC CAGCGGTGGT
4621 TTGTTTGCCG GATCAAGAGC TACCAACTCT TTTTCCGAAG GTAACGGCT TCAGCAGAGC
4681 GCAGATACCA AATACTGTCC TTCTAGTGTA GCCGTAGTTA GGCCACCACT TCAAGAATC
4741 TGTAGCACCG CCTACATACC TCGCTCTGCT AATCCTGTTA CCAGTGGCTG CTGCCAGTGG
4801 CGATAAGTCG TGTCTTACCG GGTGAGACTC AAGACGATAG TTACCGGATA AGGCGCAGCG
4861 GTCGGGCTGA ACGGGGGGTT CGTGACACA GCCCAGCTTG GAGCGAACGA CCTACACCGA
4921 ACTGAGATAC CTACAGCGTG AGCTATGAGA AAGCGCCACG CTTCCCGAAG GGAGAAAGGC
4981 GGACAGGTAT CCGGTAAGCG GCAGGGTCGG AACAGGAGAG CGCACGAGG AGCTTCCAGG
5041 GGGAAACGCC TGGTATCTTT ATAGTCTGT CCGGTTTCGC CACCTCTGAC TTGAGCGTCG
5101 ATTTTGTGA TGCTCGTCAG GGGGGCGGAG CCTATGGAAA AACGCCAGCA ACGCGGCCTT
5161 TTTACGGTTC CTGGCCTTTT GCTGGCCTTT TGCTCACATG TTCTTTCCTG CGTTATCCCC
5221 TGATTCTGTG GATAACCGTA TTACCGCCTT TGAGTGAGCT GATACCGCTC GCCGAGCCG
5281 AACGACCGAG CGCAGCGAGT CAGTGAGCGA GGAAGCGGAA GAGCGCCTGA TGCGGTATTT
5341 TCTCCTTACG CATCTGTGCG GTATTTTACA CCGCATATAT GGTGCACTCT CAGTACAATC
5401 TGCTCTGATG CCGCATAGTT AAGCCAGTAT ACACCTCCGT ATCGCTACGT GACTGGGTCA
5461 TGGCTGCGCC CCGACACCCG CCAACACCCG CTGACGCGCC CTGACGGGCT TGTCTGCTCC
5521 CGGATCCGC TTACAGACAA GCTGTGACCG TCTCCGGGAG CTGCAATGTT CAGAGGTTTT
5581 CACCGTCAAT ACCGAAACGC GCGAGGCAGC TGCGGTAAAG CTCATCAGCG TGGTCGTGAA
5641 GCGATTACCA GATGTCTGCC TGTTTCATCCG CGTCCAGCTC GTTGAGTTTC TCCAGAAGCG
5701 TTAATGTCTG GCTTCTGATA AAGCGGGCCA TGTTAAGGGC GGTTTTTTCC TGTTTGGTCA
5761 CTGATGCCTC CGTGTAGGGG GGATTTCTGT TCATGGGGGT AATGATACCG ATGAAACGAG
5821 AGAGGATGCT CACGATACGG GTTACTGATG ATGAACATGC CCGGTTACTG GAACGTTGTG
5881 AGGGTAAACA ACTGGCGGTA TGGATGCGGC GGGACCAGAG AAAAATCACT CAGGGTCAAT
5941 GCCAGCGCTT CGTTAATACA GATGTAGGTG TTCCACAGGG TAGCCAGCAG CATCCTGCGA
6001 TGCAGATCCG GAACATAATG GTGCAGGGCG CTGACTTCCG CGTTTCCAGA CTTTACGAAA
6061 CACGGAAACC GAAGACCATT CATGTTGTTG CTCAGGTCGC AGACGTTTTG CAGCAGCAGT
6121 CGCTTCACGT TCGCTCGCGT ATCGGTGATT CATTCTGCTA ACCAGTAAGG CAACCCCGCC
6181 AGCCTAGCCG GGTCTCTAAC GACAGGAGCA CGATCATGCG CACCCGTGGC CAGGACCCAA
6241 CGCTGCCCGA GATGCGCCGC GTGCGCTGC TGGAGATGGC GGACGCGATG GATATGTTCT-

FIGURE 45C

131/240

6301 GCCAAGGGTT GGTTCGCGCA TTCACAGTTC TCCGCAAGAA TTGATTGGCT CCAATTCCTG
6361 GAGTGGTGAA TCCGTTAGCG AGGTGCCGCC GGCTTCCATT CAGGTCGAGG TGGCCCGGCT
6421 CCATGCACCG CGACGCAACG CGGGGAGGCA GACAAGGTAT AGGGCGGGCG CTACAATCCA
6481 TGCCAACCCG TTCCATGTGC TCGCCGAGGC GGCATAAATC GCCGTGACGA TCAGCGGTCC
6541 AGTGATCGAA GTTAGGCTGG TAAGAGCCGC GAGCGATCCT TGAAGCTGTC CCTGATGGTC
6601 GTCATCTACC TGCCTGGACA GCATGGCCTG CAACGCGGGC ATCCCGATGC CG

FIGURE 45D

132/240

FIGURE 46A

pDEST26 His6 Amino Fusion in pCMV Sport-neo Vector

600 ttg acg tca atg gga gtt tgt ttt ggc ace aaa atc aac ggg act ttc caa
 aac tgc agt tac cct caa aca aaa ccg tgg ttt tag ttg ccc tga aag gtt

651 aat gtc gta aca act cag ccc oat tga cgc aaa tgg gcg gta ggc gtg tac
 tta cag cat tgt tga ggc ggg gta act gcg ttt apc cgc cat ccg cac atg

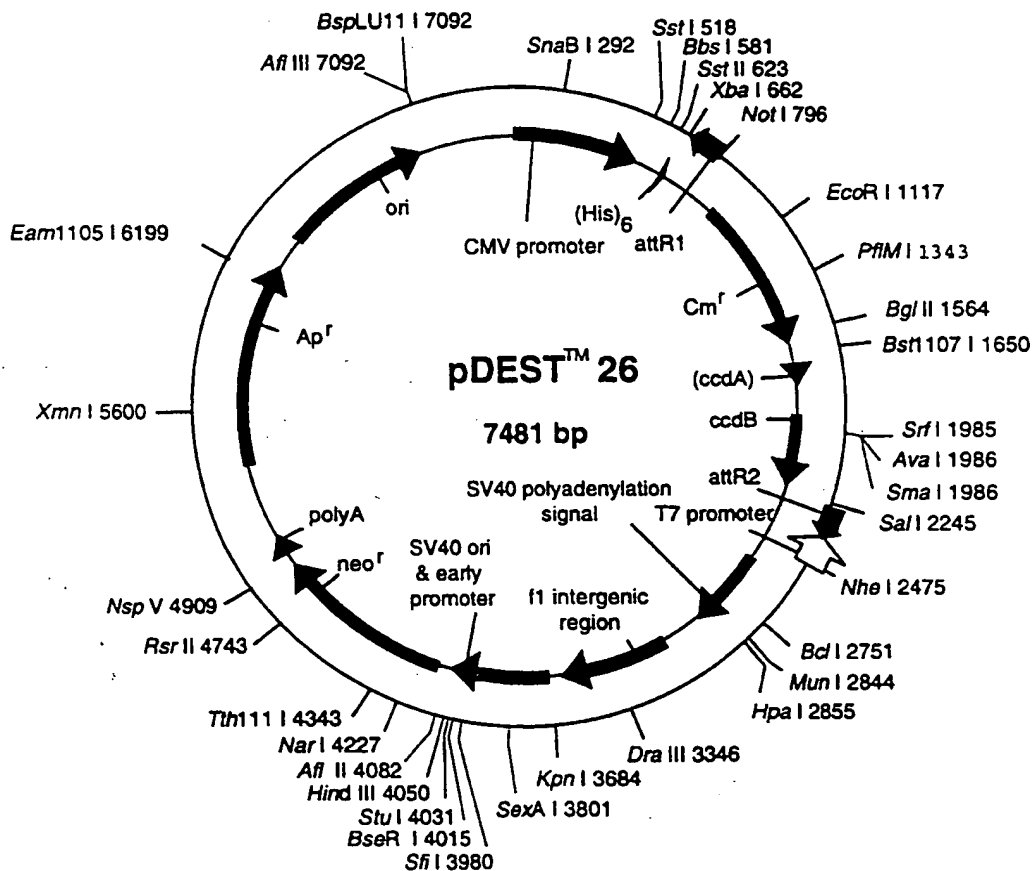
702 // ggt ggg agg tct ata taa gca gag etc gtt tag tga acc gtc aga tgg ttt
 // cca ccc tcc aga tat att cgt ctc gag caa atc act tgg cag tct agc gga

753 gga gac gtc atc cac gct gtt ttg acc tcc ata gaa gac acc ggg acc gat
 cct ctg cgg tag gtg cga caa aac tgg agg tat ctt ctg tgg ccc tgg cta

804 cca gcc tcc gga etc tag cct agg ccg cgg acc atg gcg tac tac cat cac
 ggt cgg agg cct gag atc gga tcc gcc gcc tgg tac cgc atg atg gta gtc

855 H H H H S R S T S L V K K A
 cat cac cat cac tct aga tca aca agt ttg tac aaa aaa gct gaa cga gaa
 gta gtg gta gtg aga tct agt tgt tca aac atg ttt ttt cga ctt gct ctt

Start Transl
Int



pDEST26 7481 bp

<u>Location (Base Nos.)</u>	<u>Gene Encoded</u>
492..509	his6
619..519	attR1
752..1411	CmR
1531..1615	inactivated ccdA
1753..2058	ccdB
2099..2223	attR2
2409..2771	SV40 polyA
2966..3421	f1 intergenic region
3485..3903	SV40 promoter
3948..4742	neo
4806..4854	polyA
5265..6125	Apr
6274..6913	ori
7344..385	CMV promoter

1	GTAAACTGCC	CACTTGGCAG	TACATCAAGT	GTATCATATG	CCAAGTACGC	CCCCATTATGA
61	CGTCAATGAC	GGTAAATGGC	CCGCCTGGCA	TTATGCCAG	TACATGACCT	TATGGGACTT
121	TCCTACTTGG	CAGTACATCT	ACGTATTAGT	CATCGCTATT	ACCATGGTGA	TGCGGTTTTG
181	GCAGTACATC	AATGGGCGTG	GATAGCGGTT	TGACTCACGG	GGATTTCCTAA	GTCTCCACCC
241	CATTGACGTC	AATGGGAGTT	TGTTTTGGCA	CCAAAATCAA	CGGGACTTTC	CAAAATGTCTG
301	TAACAACTCC	GGCCCATTTGA	CGCAAATGGG	CGGTAGGCGT	GTACGGTGGG	AGGTCTATAT
361	AAGCAGAGCT	CGTTTAGTGA	ACCGTCAGAT	CGCCTGGAGA	CGCCATCCAC	GCTGTTTTGA
421	CCTCCATAGA	AGACACCGGG	ACCGATCCAG	CCTCCGGACT	CTAGCCTAGG	CCGCGGACCA
481	TGGCGTACTA	CCATCACCAT	CACCATCACT	CTAGATCAAC	AAGTTTGATC	AAAAAAGCTG
541	AACGAGAAAC	GTAAAATGAT	ATAAATATCA	ATATATTAAA	TTAGATTTTG	CATAAAAAAAC
601	AGACTACATA	ATACTGTAAA	ACACAACATA	TCCAGTCACT	ATGGCGGCCG	CATTAGGCAC
661	CCCAGGCTTT	ACACTTTATG	CTTCCGGCTC	GTATAATGTG	TGGATTTTGA	GTAGGATCC
721	GGCGAGATTT	TCAGGAGCTA	AGGAAGCTAA	AATGGAGAAA	AAAATCACTG	GATATACCAC
781	CGTTGATATA	TCCCAATGGC	ATCGTAAAGA	ACATTTTGAG	GCATTTTCAGT	CAGTTGCTCA
841	ATGTACCTAT	AACCAGACCG	TTCAGCTGGA	TATTACGGCC	TTTTTAAAGA	CCGTAAAGAA
901	AAATAAGCAC	AAGTTTATC	CGGCCTTTAT	TCACATTCTT	GCCCGCCTGA	TGAATGCTCA
961	TCCGGAATTC	CGTATGGCAA	TGAAAGACGG	TGAGCTGGTG	ATATGGGATA	GTGTTCACCC
1021	TTGTTACACC	GTTTTCCATG	AGCAAATGTA	AACGTTTTTC	TCGCTCTGGA	GTGAATACCA
1081	CGACGATTTT	CGGCAGTTTC	TACACATATA	TTCGCAAGAT	GTGGCGTGTT	ACGGTGAAAA
1141	CCTGGCCTAT	TTCCCTAAAG	GGTTTATTGA	GAATATGTTT	TTCGTCTCAG	CCAATCCCCTG
1201	GGTGAGTTTC	ACCAGTTTTG	ATTTAAACGT	GGCCAATATG	GACAACTTCT	TCGCCCCCGT
1261	TTTCAACCATG	GGCAAATATT	ATACGCAAGG	CGACAAGGTG	CTGATGCCGC	TGGCGATTCA
1321	GGTTCATCAT	GCCGTCTGTG	ATGGCTTCCA	TGTCGGCAGA	ATGCTTAATG	AATTACAACA
1381	GTAATGCGAT	GAGTGGCAGG	GCGGGGCGTA	AAGATCTGGA	TCCGGCTTAC	TAAAAGCCAG
1441	ATAACAGTAT	GCGTATTGTC	GCGCTGATTT	TTGCGGTATA	AGAATATATA	CTGATATGTA
1501	TACCCGAAGT	ATGTCAAAAA	GAGGTGTGCT	ATGAAGCAGC	GTATTACAGT	GACAGTTGAC
1561	AGCGACAGCT	ATCAGTTGCT	CAAGGCATAT	ATGATGTCAA	TATCTCCGGT	CTGGTAAGCA
1621	CAACCATGCA	GAATGAAGCC	CGTCGTCTGC	GTGCCGAACG	CTGGAAAGCG	GAAAATCAGG
1681	AAGGGATGGC	TGAGGTCGCC	CGGTTTATTG	AAATGAACGG	CTCTTTTGCT	GACGAGAACA
1741	GGGACTGGTG	AAATGCAGTT	TAAGGTTTAC	ACCTATAAAA	GAGAGAGCCG	TTATCGTCTG
1801	TTTGTTGGATG	TACAGAGTGA	TATTATTGAC	ACGCCCGGGC	GACGGATGGT	GATCCCCCTG
1861	GCCAGTGCAC	GTCTGCTGTC	AGATAAAGTC	TCCCGTGAAC	TTTACCCGGT	GGTGCATATC
1921	GGGGATGAAA	GCTGGCGCAT	GATGACCACC	GATATGGCCA	GTGTGCCGGT	CTCCGTTATC
1981	GGGGAAGAAG	TGGCTGATCT	CAGCCACCGC	GAAAATGACA	TCAAAAACGC	CATTAACTTG
2041	ATGTTCTGGG	GAATATAAAT	GTCAGGCTCC	CTTATACACA	GCCAGTCTGC	AGGTGACACA
2101	TAGTCACTGG	ATATGTTGTG	TTTTACAGTA	TTATGTAGTC	TGTTTTTTAT	GCAAAATCTA
2161	ATTTAATATA	TTGATATTTA	TATCATTTTA	CGTTTCTCGT	TCAGCTTCTT	TGTACAAAGT
2221	GGTTGATCGC	GTGCATGCGA	CGTCATAGCT	CTCTCCCTAT	AGTGAGTCGT	ATTATAAGCT
2281	AGGCACTGGC	CGTCGTTTTA	CAACGTCGTG	ACTGGGAAAA	CTGCTAGCTT	GGGATCCTTG -

134/240

2341 TGAAGGAACC TTA CTCTCTGT GGTGTGACAT AATTGGACAA ACTACCTACA GAGATTTAAA
2401 GCTCTAAGGT AAATATAAAA TTTTAAAGTG TATAATGTGT TAAACTAGCT GCATATGCTT
2461 GCTGCTTGAG AGTTTTGCTT ACTGAGTATG ATTTATGAAA ATATTATACA CAGGAGCTAG
2521 TGATTCTAAT TGTTTGTGTA TTTTAGATTC ACAGTCCCAA GGCTCATTTC AGGCCCTCA
2581 GTCCTCACAG TCTGTTTCATG ATCATAATCA GCCATACCAC ATTTGTAGAG GTTTTACTTG
2641 CTTTAAAAAA CCTCCACAC CTCCCCCTGA ACCTGAAACA TAAAATGAAT GCAATTGTTG
2701 TTGTTAACTT GTTTATTGCA GCTTATAATG GTTACAAATA AAGCAATAGC ATCACAAATT
2761 TCACAAATAA AGCATTTTTT TCACTGCATT CTAGTTGTGG TTTGTCCAAA CTCATCAATG
2821 TATCTTATCA TGTCTGGATC GATCCTGCAT TAATGAATCG GCCAACGCGC GGGGAGAGGC
2881 GGTTCGCGTA TTGGCTGGCG TAATAGCGAA GAGGCCCGCA CCGATCGCCC TTCCCAACAG
2941 TTGCGCAGCC TGAATGGCGA ATGGGACGCG CCCTGTAGCG GCGCATTAAG CGCGGCGGGT
3001 GTGGTGGTTA CGCGCAGCGT GACCGCTACA CTTGCCAGCG CCCTAGCGCC CGCTCCTTTC
3061 GCTTCTTCC CTTCCTTTCT CGCCACGTTT GCCGGCTTTC CCGTCAAGC TCTAAATCGG
3121 GGGCTCCCTT TAGGGTCCG ATTTAGTGCT TTACGGCACC TCGACCCCAA AAAAATTGAT
3181 TAGGGTGATG GTTCACGTAG TGGGCCATCG CCCTGATAGA CGGTTTTTCG CCCTTTGACG
3241 TTGGAGTCCA CGTCTTTTAA TAGTGGACTC TTGTTCCAAA CTGGAACAAC ACTCAACCCT
3301 ATCTCGGTCT ATTCTTTTGA TTTATAAGGG ATTTTGCCGA TTTCCGCCCTA TTGGTTAAAA
3361 AATGAGCTGA TTTAACAAT ATTTAACGCG AATTTTAAACA AAATATTAAC GTTTACAATT
3421 TCGCTGATG CGGTATTTTC TCCTTACGCA TCTGTGCGGT ATTTACACC GCATACGCGG
3481 ATCTGCGCAG CACCATGGCC TGAAATAACC TCTGAAAGAG GAACTTGSTT AGGTACCTTC
3541 TGAGGCGGAA AGAACCAGCT GTGGAATGTG TGTCAAGTTAG GGTGTGGAAA GTCCCCAGGC
3601 TCCCCAGCAG GCAGAAGTAT GCAAAGCATG CATCTCAATT AGTCAGCAAC CAGGTGTGGA
3661 AAGTCCCCAG GCTCCCCAGC AGGCAGAAGT ATGCAAAGCA TGCATCTCAA TTAGTCAGCA
3721 ACCATAGTCC CGCCCCTAAC TCCGCCATC CCGCCCCTAA CTCCGCCAG TTCCGCCCAT
3781 TCTCCGCCCC ATGGCTGACT AATTTTTTTT ATTTATGCAG AGGCCGAGGC CGCTCGGCC
3841 TCTGAGCTAT TCCAGAAGTA GTGAGGAGGC TTTTTTGAG GCCTAGGCTT TTGCAAAAAG
3901 CTTGATTCTT CTGACACAAC AGTCTCGAAC TTAAGGCTAG AGCCACCATG ATTGAACAAG
3961 ATGGATTGCA CGCAGGTTCT CCGGCCGCTT GGGTGGAGAG GCTATTCCGG TATGACTGGG
4021 CACAACAGAC AATCGGCTGC TCTGATGCCG CCGTGTTCCG GCTGTACGCG CAGGGGCGCC
4081 CGGTTCTTTT TGTCAAGACC GACCTGTCCG GTGCCCTGAA TGAAGTGCAG GACGAGGCAG
4141 CGCGGCTATC GTGGCTGGCC ACGACGGGCG TTCCTTGCGC AGCTGTGCTC GACGTTGTCA
4201 CTGAAGCGGG AAGGGACTGG CTGCTATTGG GCGAAGTGCC GGGGCAGGAT CTCCTGTCAT
4261 CTCACCTTGC TCCTGCCGAG AAAGTATCCA TCATGGCTGA TGCAATGCGG CGGCTGCATA
4321 CGCTTGATCC GGCTACCTGC CCATTCGACC ACCAAGCGAA ACATCGCATC GAGCGAGCAC
4381 GTACTCGGAT GGAAGCCGGT CTTGTGATCT AGGATGATCT GGACGAAGAG CATCAGGGGC
4441 TCGCGCCAGC CGAACTGTTT GCCAGGCTCA AGGCGCGCAT GCCCGACGGC GAGGATCTCG
4501 TCGTGACCCA TGGCGATGCC TGCTTGCCGA ATATCATGGT GGAAAATGGC CGCTTTTCTG
4561 GATTTCATCGA CTGTGCGCGG CTGGGTGTGG CGGACCGCTA TCAGGACATA GCGTTGGCTA
4621 CCCGTGATAT TGCTGAAGAG CTTGGCGGCG AATGGGCTGA CCGCTTCTC GTGCTTTACG
4681 GTATCGCCGC TCCCATTTCG CAGCGCATCG CCTTCTATCG CCTTCTGAC GAGTCTTCT
4741 GAGCGGGACT CTGGGGTTTCG AAATGACCGA CCAAGCGACG CCCAACCTGC CATCAGCATG
4801 GCCGCAATAA AATATCTTTA TTTTCATTAC ATCTGTGTGT TGGTTTTTTG TGTGAATCGA
4861 TAGCGATAAG GATCCGCGTA TGGTGCACTC TCAGTACAAT CTGCTCTGAT GCCGCATAGT
4921 TAAGCCAGCC CCGACACCCG CCAACACCCG CTGACGCGCC CTGACGGGCT TGTCTGCTCC
4981 CGGCATCCGC TTACAGACAA GCTGTGACCG TCTCCGGGAG CTGCATGTGT CAGAGGTTTT
5041 CACCGTCATC ACCGAAACGC GCGAGACGAA AGGGCCTCGT GATACGCCCTA TTTTATAGG
5101 TTAATGTCAT GATAATAATG GTTTCTTAGA CGTCAGGTGG CACTTTTTCG GGAATGTGC
5161 GCGGAACCCC TATTTGTTTA TTTTCTAAA TACATTCAA TATGTATCCG CTCATGAGAC
5221 AATAACCTG ATAAATGCTT CAATAATATT GAAAAAGGAA GAGTATGAGT ATTCACATT
5281 TCCGTGTGCG CCTATTCCC TTTTTTGCGG CATTTTGCCT TCCTGTTTTT GCTACCCAG
5341 AAACGCTGGT GAAAGTAAAA GATGCTGAAG ATCAGTTGGG TGCACGAGTG GGTACATCG
5401 AACTGGATCT CAACAGCGGT AAGATCCTTG AGAGTTTTCG CCCCAGAGAA CGTTTTCCAA
5461 TGATGAGCAC TTTTAAAGTT CTGCTATGTG GCGCGGTATT ATCCCGTATT GACGCCGGC
5521 AAGAGCAACT CGGTCGCCGC ATACACTATT CTCAGAATGA CTTGGTTGAG TACTACCAG
5581 TCACAGAAAA GCATCTTACG GATGGCATGA CAGTAAGAGA ATTATGCAGT GCTGCCATAA
5641 CCATGAGTGA TAACACTGCG GCCAATTAC TTCTGACAAC GATCGGAGGA CCGAAGGAGC
5701 TAACCGCTTT TTTGCACAAC ATGGGGGATC ATGTAACCTG CCTTGATCGT TGGGAACCGG
5761 AGCTGAATGA AGCCATACCA AACGACGAGC GTGACACCAC GATGCCTGTA GCAATGGCAA

FIGURE 46C

5821 CAACGTTGCG CAAACTATTA ACTGGCGAAC TACTTACTCT AGCTTCCCGG CAACAATTAA
5881 TAGACTGGAT GGAGGCGGAT AAAGTTGCAG GACCACTTCT GCGCTCGGCC CTTCGGCTG
5941 GCTGGTTTAT TGCTGATAAA TCTGGAGCCG GTGAGCGTGG GTCTCGCGGT ATCATTGCAG
6001 CACTGGGGCC AGATGGTAAG CCCTCCCGTA TCGTAGTTAT CTACACGACG GGGAGTCAGG
6061 CAACTATGGA TGAACGAAAT AGACAGATCG CTGAGATAGG TGCCTCACTG ATTAAGCATT
6121 GGTAAGTCTC AGACCAAGTT TACTCATATA TACTTTAGAT TGATTTAAAA CTTCATTTTT
6181 AATTTAAAAG GATCTAGGTG AAGATCCTTT TTGATAATCT CATGACCAAA ATCCCTTAAC
6241 GTGAGTTTTT GTTCCACTGA GCGTCAGACC CCGTAGAAAA GATCAAAGGA TCTTCTTGAG
6301 ATCCTTTTTT TCTGCGCGTA ATCTGCTGCT TGCAAACAAA AAAACCACCG CTACCAGCGG
6361 TGGTTTGT TT GCGGATCAA GAGCTACCAA CTCTTTTCC GAAGGTAAC GGCTTCAGCA
6421 GAGCGCAGAT ACCAAATACT GTCTTCTAG TGTAGCCGTA GTTAGGCCAC CACTTCAAGA
6481 ACTCTGTAGC ACCGCCTACA TACCTCGCTC TGCTAATCCT GTTACCAGTG GCTGCTGCCA
6541 GTGGCGATAA GTCGTGTCTT ACCGGGTTGG ACTCAAGACG ATAGTTACCG GATAAGGCGC
6601 AGCGGTCGGG CTGAACGGGG GGTTCGTGCA CACAGCCCAG CTGAGAGCGA ACGACCTACA
6661 CCGAACTGAG ATACCTACAG CGTGAGCATT GAGAAAGCGC CACGCTTCCC GAAGGGAGAA
6721 AGGCGGACAG GTATCCGTA AGCGGCAGGG TCGGAACAGG AGAGCGCACG AGGGAGCTTC
6781 CAGGGGGAAA CGCCTGGTAT CTTTATAGTC CTGTGCGGTT TCGCCACCTC TGACTTGAGC
6841 GTCGATTTTT GTGATGCTCG TCAGGGGGGC GGAGCCTATG GAAAAACGCC AGCAACGCGG
6901 CCTTTTACG GTTCCCTGGCC TTTTGCTGGC CTTTGTCTCA CATGTTCTTT CTGCGTTAT
6961 CCCCTGATTC TGTGGATAAC CGTATTACCG CCTTTGAGTG AGCTGATACC GCTCGCCGCA
7021 GCCGAACGAC CGAGCGCAGC GAGTCAGTGA GCGAGGAAGC GGAAGAGCGC CCAATACGCA
7081 AACCGCCTCT CCCCGCGCGT TGGCCGATTC ATTAATGCAG AGCTTGCAAT TCGCGCGTTT
7141 TTCAATATTA TTGAAGCATT TATCAGGGTT ATTGTCTCAT GAGCGGATAC ATATTTGAAT
7201 GTATTTAGAA AAATAAACAA ATAGGGGTTT CGCGCACATT TCCCCGAAAA GTGCCACCTG
7261 ACGTCTAAGA AACCATTATT ATCATGACAT TAACCTATAA AAATAGGCGT AGTACGAGGC
7321 CCTTCACTC ATTAGATGCA TGTCGTTACA TAACTTACGG TAAATGGCCC GCCTGGCTGA
7381 CCGCCCAACG ACCCCCGCCC ATTGACGTCA ATAATGACGT ATGTTCCCAT AGTAACGCCA
7441 ATAGGGACTT TCCATTGACG TCAATGGGTG GAGTATTAC G

136/240
FIGURE 47A

pDEST 27 GST Amino Fusion in pCMV Sport-neo Vector

CMV Promoter

600 // nac ggt ggg agg tct ata taa gca gag ctc gtt tag tga acc gtc aga tcy
 // ntg cca ccc tcc aga tat att cgt ctc gag caa atc act tgg dag tct agc

651 cct gga gac gcc atc cac gct gtt ttg acc tcc ata gaa gac acc ggg acc
 gga cct ctg cgg tag gtg cga caa aac tgg agg tat ctt ctg tgg ccc tgg

702 gat cca gcc tcc gga ctc tag cct agg cgg cgg acc atg gcc cct ata cta
 cta ggt cgg agg cct gag atc gga tcc ggc gcc tgg tag cgg gga tat gat
 Start Translating GST

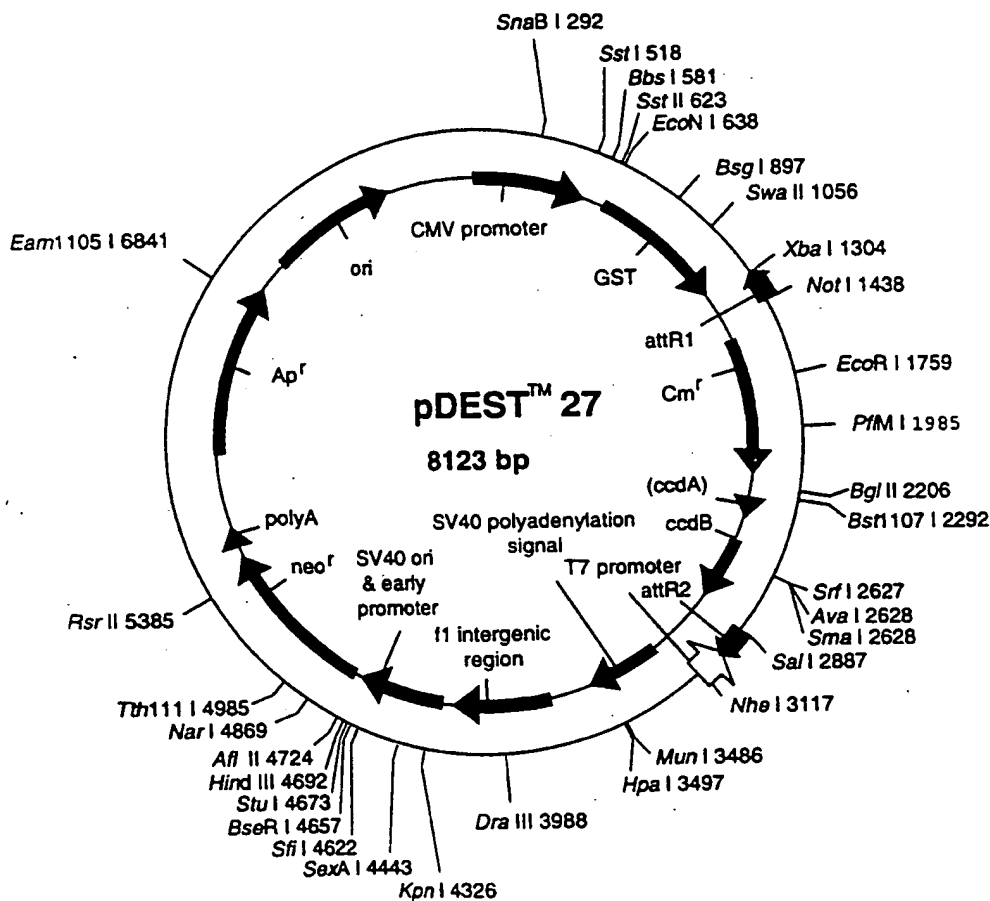
753 ggt tat tgg aaa att aag ggc ctt gtg caa ccc acc cga ctt ctt ttg gaa
 cca ata acc ttt taa ttc ccg gaa cac gtt ggg tga gct gaa gaa aac ctt

804 tat ctt gaa gaa aaa tat gaa gag cat ttg tat gag cgc gat gaa ggt gat
 ata gaa ctt ctt ttt ata ctt ctc gta aac ata ctc gcg cta ctt cca cta

1365 // ttt ggt ggt ggc gac cat cct cca aaa tgc gat ctg gtt ccg cgt tct aga
 // aaa cca cca ccg ctg gta gga ggt ttt agc cta gac caa ggc gca aga tct

1416 tca aca agt ttg tac aaa aaa gct gaa cga gaa acg
 agt tgt tca aac atg ttt ttt cga ctt gct ctt tgc

Int attR1



137/240

pDEST27 8123 bp (rotated to position 7800)

<u>Location (Base Nos.)</u>	<u>Gene Encoded</u>
130..793	GST
803..927	attR1
1036..1695	CmR
1815..1899	inactivated ccdA
2037..2342	ccdB
2383..2507	attR2
2693..3055	SV40 polyA
3250..3705	f1 intergenic region
3769..4187	SV40 promoter
4232..5026	neo
5090..5138	polyA
5549..6409	Apr
6558..7197	ori
7628..27	CMV promoter

```

1 ATAAGCAGAG CTCGTTTAGT GAACCGTCAG ATCGCCTGGA GACGCCATCC ACGCTGTTTT
61 GACCTCCATA GAAGACACCG GGACCGATCC AGCCTCCGGA CTCTAGCCTA GGCCGCGGAC
121 CATGGCCCCCT AACTAGGTT ATTGGAATAA TAAGGGCCTT GTGCAACCCA CTCGACTTCT
181 TTTGGAATAT CTTGAAGAAA AATATGAAGA GCATTTGTAT GAGCGCGATG AAGGTGATAA
241 ATGGCGAAAC AAAAAGTTTG AATTGGGTTT GGAGTTTCCC AATCTTCCTT ATTATATTGA
301 TGGTGATGTT AAATTAACAC AGTCTATGGC CATCATACGT TATATAGCTG ACAAGCACAA
361 CATGTTGGGT GGTGTCCAA AAGAGCGTGC AGAGATTTC AATGCTTGAAG GAGCGGTTTT
421 GGATATTAGA TACGGTGTTC CGAGAATTGC ATATAGTAAA GACTTTGAAA CTCTCAAAGT
481 TGATTTTCTT AGCAAGCTAC CTGAAATGCT GAAAATGTTT GAAGATCGTT TATGTCATAA
541 AACATATTTA AATGGTGATC ATGTAACCCA TCCTGACTTC ATGTTGTATG ACGCTCTTGA
601 TGTTGTTTTA TACATGGACC CAATGTGCCT GGATGCGTTC CCAAAATTAG TTTGTTTTAA
661 AAAACGTATT GAAGCTATCC CACAAATTGA TAAGTACTTG AAATCCAGCA AGTATATAGC
721 ATGGCCTTTG CAGGGCTGGC AAGCCACGTT TGGTGGTGGC GACCATCCTC CAAAATCGGA
781 TCTGGTTCGG CGTTCTAGAT CAACAAGTTT GTACAAAAAA GCTGAACGAG AAACGTAAAA
841 TGATATAAAT ATCAATATAT TAAATTAGAT TTTGCATAAA AAACAGACTA CATAATACTG
901 TAAACACAA CATATCCAGT CACTATGGCG GCCGCATTAG GCACCCAGG CTTTACACTT
961 TATGCTTCCG GCTCGTATAA TGTGTGGATT TTGAGTTAGG ATCCGGCGAG ATTTTCAGGA
1021 GCTAAGGAAG CTAATATGGA GAAAAAATC ACTGGATATA CCACCGTTGA TATATCCCAA
1081 TGGCATCGTA AAGAACATTT TGAGGCATTT CAGTCAGTTG CTCAATGTAC CTATAACCGA
1141 ACCGTTTCAGC TGGATATTAC GGCCTTTTAA AAGACCGTAA AGAAAAATAA GCACAAGTTT
1201 TATCCGGCCT TTATTCACAT TCTTGCCCGC CTGATGAATG CTCATCCGGA ATTCCGTATG
1261 GCAATGAAAG ACGGTGAGCT GGTGATATGG GATAGTGTTC ACCCTTGTTA CACCGTTTTT
1321 CATGAGCAAA CTGAAACGTT TTCATCGCTC TGGAGTGAAT ACCACGACGA TTTCCGGCAG
1381 TTTCTACACA TATATTCGCA AGATGTGGCG TGTTACGGTG AAAACCTGGC CTATTTCCCT
1441 AAAGGGTTTA TTGAGAATAT GTTTTTTCGC TCAGCCAAAT CCTGGGTGAG TTTCAACAGT
1501 TTTGATTAA ACGTGGCCAA TATGGACAAC TTCTTCGCCC CCGTTTTTAC CATGGGCAAA
1561 TATTATACGC AAGGCGACAA GGTGCTGATG CCGCTGGCGA TTCAGGTTCA TCATGCCGTC
1621 TGTGATGGCT TCCATGTCGG CAGAATGCTT AATGAATTAC AACAGTACTG CGATGAGTGG
1681 CAGGGCGGGG CGTAAAGATC TGGATCCGGC TTAATAAAG CCAGATAACA GTATGCGTAT
1741 TTGCGCGCTG ATTTTTCGGG TATAAGAATA TATACTGATA TGTATACCGG AAGTATGTCA
1801 AAAAGAGGTG TGCTATGAAG CAGCGTATTA CAGTGACAGT TGACAGCGAC AGCTATCAGT
1861 TGCTCAAGGC ATATATGATG TCAATATCTC CCGTCTGGTA AGCACAACCA TGCAGAATGA
1921 AGCCCGTCTG CTGCGTGCCG AACCGTGGAA AGCGGAAAAT CAGGAAGGGA TGGCTGAGGT
1981 CGCCCGGTTT ATTGAAATGA ACGGCTCTTT TGCTGACGAG AACAGGGACT GGTGAAATGC
2041 AGTTTAAGGT TTACACCTAT AAAAGAGAGA GCCGTTATCG TCTGTTTGTG GATGTACAGA
2101 GTGATATTAT TGACACGCCC GGGCGACGGA TGGTGATCCC CCTGGCCAGT GCACGTCTGC
2161 TGTCAGATAA AGTCTCCCGT GAACCTTTACC CCGTGGTGCA TATCGGGGAT GAAAGCTGGC
2221 GCATGATGAC CACCGATATG GCCAGTGTGC CCGTCTCCGT TATCGGGGAA GAAGTGGCTG
2281 ATCTCAGCCA CCGCGAAAAT GACATCAAAA ACGCCATTAA CCTGATGTTC TGGGGAATAT-

```

Figure 47B

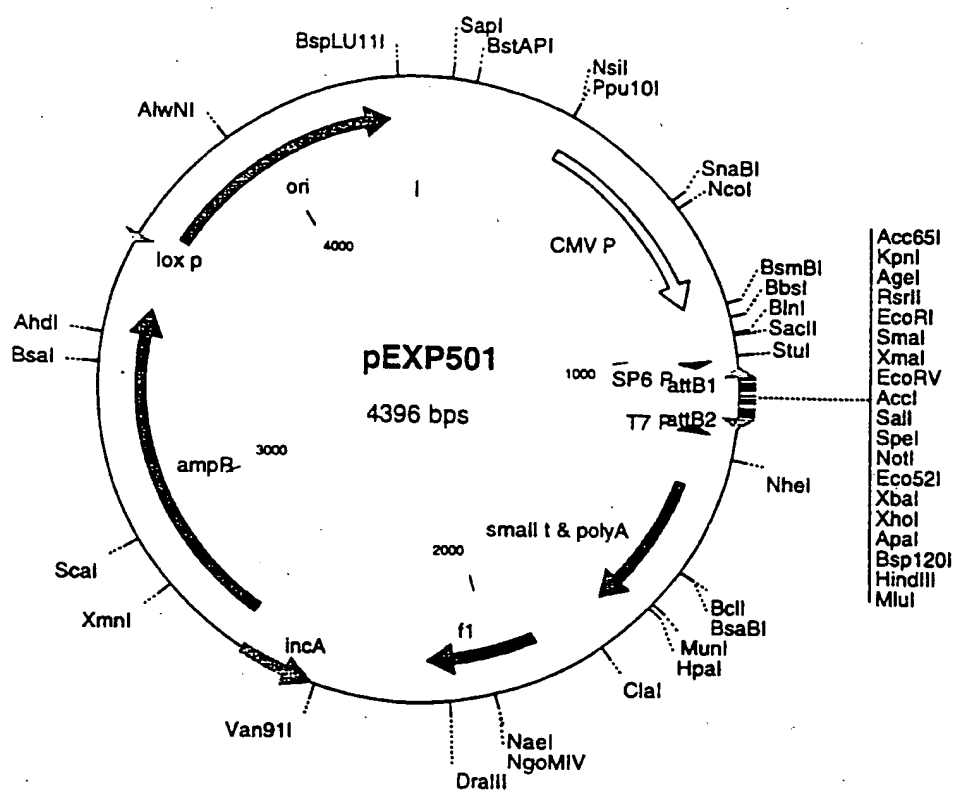
138/240

2341 AAATGTCAGG CTCCCTTATA CACAGCCAGT CTGCAGGTCG ACCATAGTGA CTGGATATGT
2401 TGTGTTTTAC AGTATTATGT AGTCTGTTTT TTATGCAAAA TCTAATTTAA TATATTGATA
2461 TTTATATCAT TTTACGTTTC TCGTTCAGTG TTCTTGTACA AAGTGTTGA TCGCGTGCAT
2521 GCGACGTCAT AGCTCTCTCC CTATAGTGAG TCGTATTATA AGCTAGGCAC TGGCCGTCGT
2581 TTTACAACGT CGTGACTGGG AAAACTGCTA GCTTGGGATC TTTGTGAAGG AACCTTACTT
2641 CTGTGGTGTG ACATAATTGG ACAAACCTACC TACAGAGATT TAAAGCTCTA AGGTAAATAT
2701 AAAATTTTTA AGTGATATA GTGTAAACT AGCTGCATAT GCTTGCTGCT TGAGAGTTTT
2761 GCTTACTGAG TATGATTTAT GAAAATATTA TACACAGGAG CTAGTGATTG TAATTGTTTG
2821 TGTATTTTAG ATTCACAGTC CCAAGGCTCA TTTAGGCCCC CTCAGTCTCT ACAGTCTGTT
2881 CATGATCATA ATCAGCCATA CCACATTTGT AGAGGTTTTA CTGCTTTAA AAAACCTCCC
2941 ACACCTCCCC CTGAACCTGA AACATAAAAT GAATGCAATT GTTGTGTTA ACTTGTATT
3001 TGCAGCTTAT AATGGTTACA AATAAAGCAA TAGCATCACA AATTTACAAA ATAAAGCATT
3061 TTTTCACTG CATTCTAGTT GTGGTTTGTG CAAACTCATC AATGTATCTT ATCATGTCTG
3121 GATCGATCCT GCATTAATGA ATCGGCCAAC GCGCGGGGAG AGGCGGTTTG CGTATTGGCT
3181 GCGGTAATAG CGAAGAGGCC CGCACCGATC GCCCTTCCCA ACAGTTGCGC AGCCTGAATG
3241 GCGAATGGGA CGCGCCCTGT AGCGGCGCAT TAAGCGCGGC GGGTGTGGTG GTTACGCGCA
3301 GCGTGACCGC TACACTTGCC AGCGCCCTAG CGCCCGCTCC TTTGCTTTT TCCCTTCCCT
3361 TTCTCGCCAC GTTCGCGGCG TTTCCCGCTC AAGCTCTAAA TCGGGGGCTC CCTTTAGGGT
3421 TCCGATTTAG TGCTTTACGG CACCTCGACC CCAAAAACT TGATTAGGGT GATGGTTTAC
3481 GTAGTGGGCC ATCGCCCTGA TAGACGGTTT TTCGCGCTTT GACGTGGAG TCACGTTCT
3541 TTAATAGTGG ACTCTTGTTT CAAACTGGAA CAACACTCAA CCCTATCTCG GTCTATTCTT
3601 TTGATTTATA AGGGATTTTG CCGATTTTCG CCTATTGGTT AAAAAATGAG CTGATTTAAC
3661 AAATATTTAA CGCGAATTTT AACAAAATAT TAACGTTTAC AATTTGCTCCT GATGCGGTAT
3721 TTTCTCCTTA CGCATCTGTG CCGTATTTCA CACCGCATAC GCGGATCTGC GCAGCACCAT
3781 GGCCTGAAAT AACCTCTGAA AGAGGAACTT GGTTAGGTAC CTTCTGAGGC GGAAAGAACC
3841 AGCTGTGGAA TGTGTGTGAG TTAGGGTGTG GAAAGTCCCC AGGCTCCCCA GCAGGCAGAA
3901 GTATGCAAAAG CATGCATCTC AATTAGTCAG CAACCAGGTG TGGAAAGTCC CCAGGCTCCC
3961 CAGCAGGCAG AAGTATGCAA AGCATGCATC TCAATTAGTC AGCAACCATA GTCCCGCCCC
4021 TAACTCCGCC CATCCCGCCC CTAACCTCCG CCAGTTCCGC CCATTCTCCG CCCCATGGCT
4081 GACTAATTTT TTTTATTTAT GCAGAGGCCG AGGCCGCTC GGCCTCTGAG CTATTCCAGA
4141 AGTAGTGAGG AGGCTTTTTT GGAGGCTTAG GCTTTTGCAA AAAGCTTGAT TCTTCTGACA
4201 CAACAGTCTC GAACCTAAGG CTAGAGCCAC CATGATTGAA CAAGATGGAT TGACAGCAGG
4261 TTCTCCGGCC GCTTGGGTGG AGAGGCTATT CGGCTATGAC TGGGCACAAC AGACAATCGG
4321 CTGCTCTGAT GCCGCCGTGT TCCGGCTGTC AGCGCAGGGG CGCCCGGTTT TTTTGTCAA
4381 GACCGACCTG TCCGGTGCCC TGAATGAACT GCAGGACGAG GCAGCGCGG TATCGTGGCT
4441 GGCCACGACG GCGCTTCTTT GCGCAGCTGT GCTCGACGTT GTCACCTGAA CGGGAAGGGA
4501 CTGGCTGCTA TTGGGCGAAG TGCCGGGGCA GGATCTCCTG TCATCTCACC TTGCTCCTGC
4561 CGAGAAAGTA TCCATCATGG CTGATGCAAT GCGGCGGCTG CACACGCTTG ATCCGGCTAC
4621 CTGCCCATTC GACCACCAAG CGAAACATCG CATCGAGCGA GCACGTACTC GGATGGAAGC
4681 CGGTCTTGTC GATCAGGATG ATCTGGACGA AGAGCATCAG GGGCTCGCGC CAGCCGAACT
4741 GTTCGCCAGG CTCAGGCGC GCATGCCCCG CGGCGAGGAT CTCGTCTGTA CCCATGGCGA
4801 TGCCTGCTTG CCGAATATCA TGGTGGAATA TGGCCGCTTT TCTGGATTCA TCGACTGTGG
4861 CCGGCTGGGT GTGGCGGACC GCTATCAGGA CATAGCGTTG GCTACCCGTG ATATTGCTGA
4921 AGAGCTTGGC GGCGAATGGG CTGACCGCTT CCTCGTGCTT TACGGTATCG CCGCTCCCGA
4981 TTCGCAGCGC ATCGCCTTCT ATCGCCTTCT TGACGAGTTC TTCTGAGCGG GACTCTGGGG
5041 TTCGAAATGA CCGACCAAGC GACGCCAAC CTGCCATCAC GATGGCCGCA ATAAATATC
5101 TTTATTTTCA TTACATCTGT GTGTTGGTTT TTTGTGTGAA TCGATAGCGA TAAGGATCCG
5161 CGTATGGTGC ACTCTCAGTA CAATCTGCTC TGATGCCGCA TAGTTAAGCC AGCCCCGACA
5221 CCCGCCAACA CCCGCTGACG CGCCCTGACG GGCTTGTCTG CTCCCGGCAT CCGCTTACAG
5281 ACAAGCTGTG ACCGCTCTCCG GGAGCTGCAT GTGTGAGAGG TTTTACCGT CATCACCAGG
5341 ACAGCGGAGA CGAAAGGGCC TCGTGATACG CCTATTTTAA TAGGTTAATG TCATGATAAT
5401 AATGGTTTCT TAGACGTCAG GTGGCACTTT TCGGGGAAAT GTGCGCGGAA CCCCTATTTG
5461 TTTATTTTTC TAAATACATT CAAATATGTA TCCGCTCATG AGACAATAAC CCTGATAAAT
5521 GCTTCAATAA TATTGAAAAA GGAAGAGTAT GAGTATTCAA CATTTCCGTG TCGCCCTTAT
5581 TCCCTTTTTT GCGGCATTTT GCCTTCTGT TTTTGCTCAC CCAGAAACGC TGGTGAAAGT
5641 AAAAGATGCT GAAGATCAGT TGGGTGCACG AGTGGGTTAC ATCGAACTGG ATCTCAACAG
5701 CGGTAAGATC CTTGAGAGTT TTCGCCCCGA AGAACGTTTT CCAATGATGA GCACTTTTAA
5761 AGTTCGTGCTA TGTGGCGCGG TATTATCCCG TATTGACGCC GGGCAAGAGC AACTCGGTG

FIGURE 47C

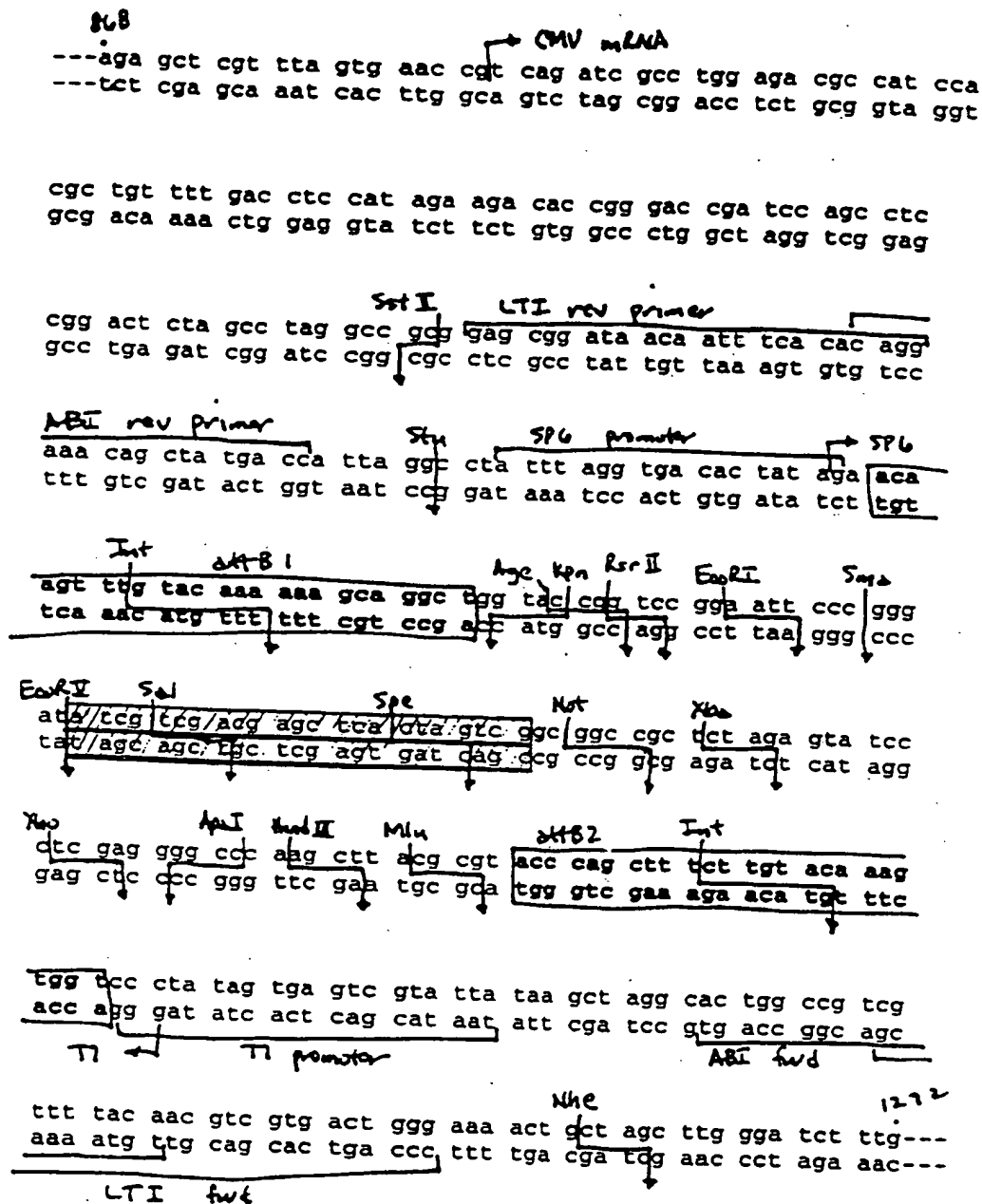
5821 CCGCATAAC TATTCTCAGA ATGACTTGGT TGAGTACTCA CCAGTCACAG AAAAGCATCT
5881 TACGGATGGC ATGACAGTAA GAGAATTATG CAGTGCTGCC ATAACCATGA GTGATAACAC
5941 TGCGGCCAAC TTACTTCTGA CAACGATCGG AGGACCGAAG GAGCTAACCG CTTTTTTGCA
6001 CAACATGGGG GATCATGTAA CTCGCCTTGA TCGTTGGGAA CCGGAGCTGA ATGAAGCCAT
6061 ACCAAACGAC GAGCGTGACA CCACGATGCC TGTAGCAATG GCAACAACGT TGCGCAAACT
6121 ATTAACCTGGC GAACTACTTA CTCTAGCTTC CCGGCAACAA TTAATAGACT GGATGGAGGC
6181 GGATAAAGTT GCAGGACCAC TTCTGCGCTC GGCCCTTCCG GCTGGCTGGT TTATTGCTGA
6241 TAAATCTGGA GCCGGTGAGC GTGGGTCTCG CGGTATCATT GCAGCACTGG GGCCAGATGG
6301 TAAGCCCTCC CGTATCGTAG TTATCTACAC GACGGGGAGT CAGGCAACTA TGGATGAACG
6361 AAATAGACAG ATCGCTGAGA TAGGTGCCTC ACTGATTAAG CATTGGTAAC TGTCAGACCA
6421 AGTTTACTCA TATATACTTT AGATTGATTT AAAAATTTCAT TTTTAATTTA AAAGGATCTA
6481 GGTGAAGATC CTTTTTGATA ATCTCATGAC CAAAATCCCT TAACGTGAGT TTTCTGTCCA
6541 CTGAGCGTCA GACCCCGTAG AAAAGATCAA AGGATCTTCT TGAGATCCTT TTTTCTGCG
6601 CGTAATCTGC TGCTTGCAAA CAAAAAACC ACCGCTACCA GCGGTGGTTT GTTTGCCGGA
6661 TCAAGAGCTA CCAACTCTTT TTCCGAAGGT AACTGGCTTC AGCAGAGCGC AGATACCAAA
6721 TACTGTCCTT CTAGTGTAGC CGTAGTTAGG CCACCACTTC AAGAAGCTCTG TAGCACCGCC
6781 TACATACCTC GCTCTGCTAA TCCTGTACC AGTGGCTGCT GCCAGTGGCG ATAAGTCGTG
6841 TCTTACCGGG TTGGACTCAA GACGATAGTT ACCGGATAAG GCGCAGCGGT CCGGCTGAAC
6901 GGGGGGTTTC TGACACACAGC CCAGCTTGGA GCGAACGACC TACACCGAAC TGAGATACCT
6961 ACAGCGTGAG CATTGAGAAA GCGCCACGCT TCCCGAAGGG AGAAAGGCGG ACAGGTATCC
7021 GGTAAGCGGC AGGGTCGGAA CAGGAGAGCG CACGAGGGAG CTTCCAGGGG GAAACGCCTG
7081 GTATCTTTAT AGTCCTGTCG GGTTTCGCCA CCTCTGACTT GAGCGTCGAT TTTTGTGATG
7141 CTCGTAGGGG GGGCGGAGCC TATGGAAAAA CGCCAGCAAC GCGGCCTTTT TACGGTTCCT
7201 GGCCTTTTGC TGGCCTTTTG CTCACATGTT CTTTCCTGCG TTATCCCCTG ATTCTGTGGA
7261 TAACCGTATT ACCGCCTTTG AGTGAGCTGA TACCGCTCGC CGCAGCCGAA CGACCGAGCG
7321 CAGCGAGTCA GTGAGCGAGG AAGCGGAAGA GCGCCCAATA CGCAAACCGC CTCTCCCCGC
7381 GCGTTGGCCG ATTCATTAAT GCAGAGCTTG CAATTGCGCG GTTTTTCAAT ATTATTGAAG
7441 CATTTATCAG GGTTATTGTC TCATGAGCGG ATACATATTT GAATGTATTT AGAAAAATAA
7501 ACAAATAGGG GTTCCGCGCA CATTCCCCG AAAAGTGCCA CCTGACGTCT AAGAAACCAT
7561 TATTATCATG ACATTAACCT ATAAAAATAG GCGTAGTACG AGGCCCTTTC ACTCATTAGA
7621 TGCATGTCGT TACATAACTT ACGGTAAATG GCGCGCCTGG CTGACCGCCC AACGACCCCC
7681 GCCCATTGAC GTCAATAATG ACGTATGTTT CCATAGTAAC GCCAATAGGG ACTTTCCATT
7741 GACGTCAATG GGTGGAGTAT TTACGGTAAA CTGCCCCACTT GGCAGTACAT CAAGTGTATC
7801 ATATGCCAAG TACGCCCCCT ATTGACGTCA ATGACGGTAA ATGGCCCCGC TGGCATTATG
7861 CCCAGTACAT GACCTTATGG GACTTTTCTA CTTGGCAGTA CATCTACGTA TTAGTCATCG
7921 CTATTACCAT GGTGATGCGG TTTTGGCAGT ACATCAATGG GCGTGGATAG CGGTTTGACT
7981 CACGGGGATT TCCAAGTCTC CACCCATTG ACGTCAATGG GAGTTTGTTC TGGCACCAAA
8041 ATCAACGGGA CTTTCCAAAA TGTCGTAACA ACTCCGCCCC ATTGACGCAA ATGGGCGGTA
8101 GCGGTGTACG GTGGGAGGTC TAT

140/240

Figure 4B A: pEXP501: pCMV.SPORT 6 host for attB Libraries

161/260

Figure 4B8: pEXP501 (cont'd). **Features of the att B cloning vector, pEXP501.** Bases within hatched area are replaced by cDNA in some LTI cDNA libraries.

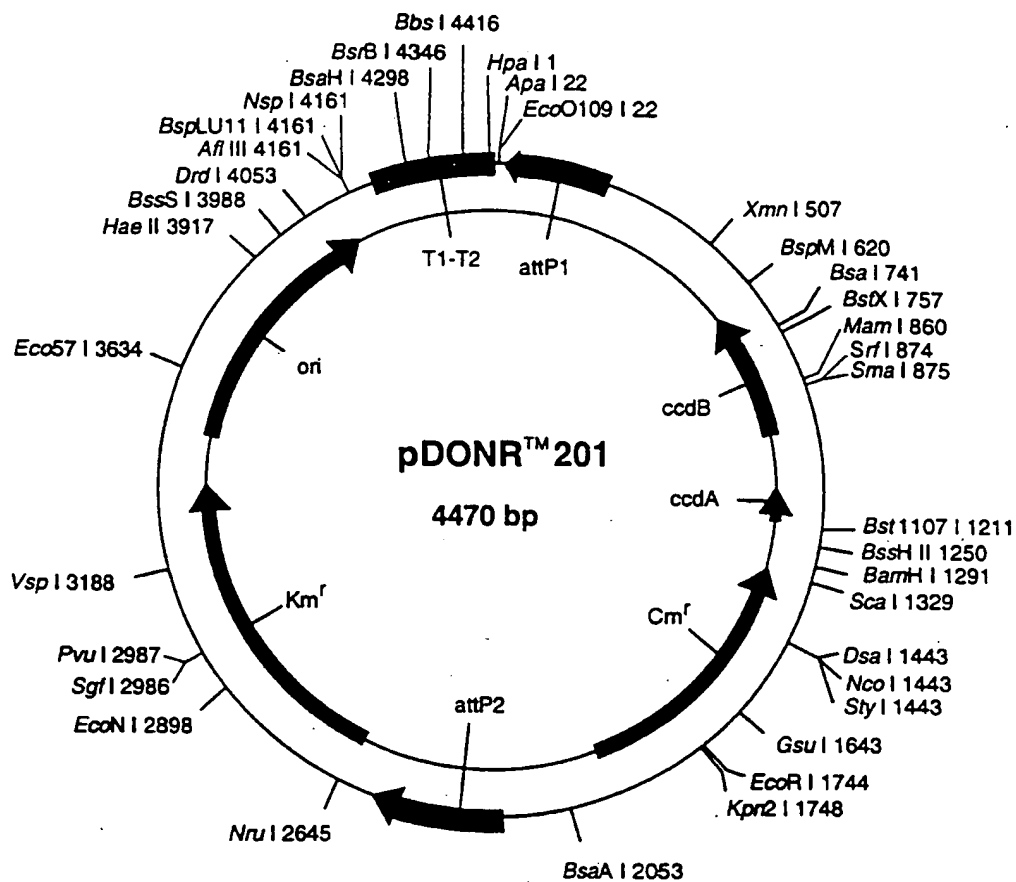


pEXP501 4396 bp

1 CCATTGCGCCA TTCAGGCTGC GCAACTGTTG GGAAGGGCGA TCGGTGCGGG CCTCTTCGCT
61 ATTACGCCAG CCAATACGCA AACCGCCTCT CCCC CGCGCT TGGCCGATTG ATTAATGCAG
121 GATCGATCCA GACATGATAA GATACATTGA TGAGTTTGGA CAAACCACAA CTAGAATGCA
181 GTGAAAAAAA TGCTTTATTT GTGAAATTTG TGATGCTATT GCTTTATTTG TAACCATTAT
241 AAGCTGCAAT AAACAAGTTA ACAACAACAA TTGCATTCTAT TTTATGTTTC AGGTTTCAGGG
301 GGAGGTGTGG GAGGTTTTTT AAAGCAAGTA AAACCTCTAC AAATGTGGTA TGGCTGATTA
361 TGATCATGAA CAGACTGTGA GGACTGAGGG GCCTGAAATG AGCCTTGGGA CTGTGAATCT
421 AAAATACACA AACAATTAGA ATCACTAGCT CCTGTGTATA ATATTTTCAT AAATCATACT
481 CAGTAAGCAA AACTCTCAAG CAGCAAGCAT ATGCAGCTAG TTTAACACAT TATACACTTA
541 AAAATTTTAT ATTTACCTTA GAGCTTTAAA TCTCTGTAGG TAGTTTGTCC AATTATGTCA
601 CACCACAGAA GTAAGGTTCC TTCACAAAGA TCCCAAGCTA GCAGTTTTC CAGTCACGAC
661 GTTGTAATAAC GACGGCCAGT GCCTAGCTTA TAATACGACT CACTATAGGG ACCACTTTGT
721 ACAAGAAAGC TGGGTACGCG TAAGCTTGGG CCCCTCGAGG GATCCTCTAG AGCGGCCGCG
781 GACTAGTGAG CTCGTCGACG ATATCCCGGG AATTCCGGAC CGGTACCAGC CTGCTTTTTT
841 GTACAAACTT GTTCTATAGT GTCACCTAAA TAGGCCTAAT GGTATAGCT GTTTCCTGTG
901 TGAAATTGTT ATCCGCTCCG CGGCCTAGGG TAGAGTCCGG AGGCTGGATC GGTCCCGGTG
961 TCTTCTATGG AGGTCAAAC AGCGTGGATG GCGTCTCCAG GCGATCTGAC GGTTCACTAA
1021 ACGAGCTCTG CTTATATAGA CCTCCACCG TACACGCCTA CCGCCCATTT GCGTCAATGG
1081 GGCGGAGTTG TTACGACATT TTGGAAGTC CCGTTGATTT TGGTGCCAAA ACAAACTCCC
1141 ATTGACGTCA ATGGGGTGA GACTTGAAA TCCCGTGAG TCAAACCGCT ATCCACGCCC
1201 ATTGATGTAC TGCCAAAACC GCATCACCAT GGTAATAGCG ATGACTAATA CGTAGATGTA
1261 CTGCCAAGTA GGAAAGTCCC ATAAGGTCAT GTACTGGGCA TAATGCCAGG CGGGCCATTT
1321 ACCGTCAATTG ACGTCAATAG GGGGCGTACT TGGCATATGA TACACTTGAT GTACTGCCAA
1381 GTGGGCAGTT TACCGTAAAT ACTCCACCCA TTGACGTCAA TGGAAAGTCC CTATTGGCGT
1441 TACTATGGGA ACATACGTCA TTATTGACGT CAATGGGCGG GGGTCGTTGG GCGGTCAGCC
1501 AGCGGGGCCA TTTACCGTAA GTTATGTAA GACATGCATC TAATGAGTGA AAGGGCCTCG
1561 TACTACGCCT ATTTTATAG GTTAATGTCA TGATAATAAT GGTTCCTTAG ACGTCAGGTG
1621 GCACCTTTTCG GGGAAATGTG CGCGGAACCC CTATTGTTT ATTTTCTAA ATACATTCAA
1681 ATATGTATCC GCTCATGAGA CAATAACCTT GATAAATGCT TCAATAATAT TGAAAAACGC
1741 GCGAATTGCA AGCTCTGCAT TAATGAATCG GCCAACGCGC GGGGAGAGGC GGTGTGCGTA
1801 TTGGGCGCTC TTCCGCTTCC TCGCTCACTG ACTCGCTGCG CTCGGTCGTT CCGGTGCGGC
1861 GAGCGGTATC AGCTCACTCA AAGGCGGTAA TACGGTTATC CACAGAATCA GGGGTAACG
1921 CAGGAAAGAA CATGTAGCA AAAGGCCAGC AAAAGGCCAG GAACCGTAAA AAGGCCGCT
1981 TGTGCGGTT TTTCCATAGG CTCCGCCCCC CTGACGAGCA TCACAAAAT CGACGCTCAA
2041 GTCAGAGGTG GCGAAACCCG ACAGGACTAT AAAGATACCA GCGGTTTCCC CCTGGAAGCT
2101 CCCTCGTGCG CTCTCCTGTT CCGACCCTGC CGCTTACCGG ATACCTGTCC GCCTTTCTCC
2161 CTTCCGGAAG CGTGGCGCTT TCTCAATGCT CACGCTGTAG GTATCTCAGT TCGGTGTAGG
2221 TCGTTCGCTC CAAGCTGGGC TGTGTGCACG AACCCCCGCT TCAGCCCGAC CGCTGCGCCT
2281 TATCCGGTAA CTATCGTCTT GAGTCCAACC CGGTAAGACA CGACTTATCG CCACTGGCAG
2341 CAGCCACTGG TAACAGGATT AGCAGAGCGA GGTATGTAGG CGGTGCTACA GAGTTCTTGA
2401 AGTGGTGGCC TAACTACGGC TACACTAGAA GGACAGTATT TGGTATCTGC GCTCTGCTGA
2461 AGCCAGTTAC CTTCCGAAAA AGAGTTGGTA GCTCTTGATC CGGCAACAA ACCACCGCTG
2521 GTAGCGGTGG TTTTGTGTT TGCAAGCAGC AGATTACGCG CAGAAAAAAA GGATCTCAAG
2581 AAGATCCTTT GATCTTTTCT ACGGGGTCTG ACGCTCAGTG GAACGAAAAC TCAGGTTAAG
2641 GGATTTTGGT CATGCCATAA CTTCTGATAG CATACTATAT ACGAAGTTAT GGCATGAGAT
2701 TATCAAAAAG GATCTTCACC TAGATCCTTT TAAATTAAAA ATGAAGTTTT AAATCAATCT
2761 AAAGTATATA TGAGTAAACT TGGTCTGACA GTTACCAATG CTTAATCAGT GAGGCACCTA
2821 TCTCAGCGAT CTGTCTATTT CGTTCATCCA TAGTTGCCTG ACTCCCGCTC GTGTAGATAA
2881 CTACGATACG GGAGGGCTTA CCATCTGGCC CCAGTGCTGC AATGATACCG CGAGACCCAC
2941 GCTCACCGGC TCCAGATTTA TCAGCAATAA ACCAGCCAGC CGGAAGGGCC GAGCGCAGAA
3001 GTGGTCCTGC AACTTTATCC GCCTCCATCC AGTCTATTAA TTGTTGCCGG GAAGCTAGAG
3061 TAAGTAGTTC GCCAGTTAAT AGTTTGCSCA ACGTTGTTGC CATTGCTACA GGCATCGTGG
3121 TGTCACGCTC GTCGTTTGGT ATGGCTTCAT TCAGCTCCGG TTCCCAACGA TCAAGGCGAG-

3181 TTACATGATC CCCCATGTTG TGCAAAAAAG CGGTTAGCTC CTCGGTCCT CCGATCGTTG
3241 TCAGAAGTAA GTTGGCCGCA GTGTTATCAC TCATGGTTAT GGCAGCACTG CATAATTCTC
3301 TTACTGTCAT GCCATCCGTA AGATGCTTTT CTGTGACTGG TGAGTACTCA ACCAAGTCAT
3361 TCTGAGAATA GTGTATGCGG CGACCGAGTT GCTCTTGCCC GGCGTCAATA CGGGATAATA
3421 CCGCGCCACA TAGCAGAACT TTAAAAGTGC TCATCATTGG AAAACGTTCT TCGGGGCGAA
3481 AACTCTCAAG GATCTTACCG CTGTTGAGAT CCAGTTCGAT GTAACCCACT CGTGCACCCA
3541 ACTGATCTTC AGCATCTTTT ACTTTCACCA GCGTTTCTGG GTGAGCAAAA ACAGGAAGGC
3601 AAAATGCCGC AAAAAAGGGA ATAAGGGCGA CACGGAAATG TTGAATACTC ATACTCTTCC
3661 TTTTCAATA TTATTGAAGC ATTTATCAGG GTTATTGTCT CATGCCAGGG GTGGGCACAC
3721 ATATTTGATA CCAGCGATCC CTACACAGCA CATAATTCAA TGCGACTTCC CTCTATCGCA
3781 CATCTTAGAC CTTTATTCTC CCTCCAGCAC ACATCGAAGC TGCCGAGCAA GCCGTTCTCA
3841 CCAGTCCAAG ACCTGGCATG AGCGGATACA TATTTGAATG TATTTAGAAA AATAAACAAA
3901 TAGGGGTTCC GCGCACATTT CCCCAGAAAAG TGCCACCTGA AATTGTAAAC GTTAATATTT
3961 TGTAAAAATT CGCGTTAAAT TTTTGTAAA TCAGCTCATT TTTTAACCAA TAGGCCGAAA
4021 TCGGCAAAAT CCCTTATAAA TCAAAAGAAT AGACCGAGAT AGGGTTGAGT GTTGTTCCAG
4081 TTTGGAACAA GAGTCCACTA TTAAAGAACG TGGACTCCAA CGTCAAAGGG CGAAAAACCG
4141 TCTATCAGGG CGATGGCCCA CTACGTGAAC CATCACCCTA ATCAAGTTTT TTGGGGTCGA
4201 GGTGCCGTAA AGCACTAAAT CGGAACCCTA AAGGGAGCCC CCGATTTAGA GCTTGACGGG
4261 GAAAGCCGGC GAACGTGGCG AGAAAGGAAG GGAAGAAAGC GAAAGGAGCG GGCGCTAGGG
4321 CGCTGGCAAG TGTAGCGGTC ACGCTGCGCG TAACCACCAC ACCCGCCGCG CTTAATGCGC
4381 CGCTACAGGG CGCGTC

FIGURE 48D



145/240

pDONR201 4470 bp (rotated to position 3516)

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
260..29		attP1
656..961		ccdB
1099..1184		ccdA
1303..1962		CmR
2210..2442		attP2
2565..3374		Kmr
3495..4134		ori

1	GTTAACGCTA	GCATGGATCT	CGGGCCCCAA	ATAATGATTT	TATTTTGACT	GATAGTGACC
61	TGTTTCGTTG	AACAAATTGA	TGAGCAATGC	TTTTTTTATA	TGCCAACTTT	GTACAAAAAA
121	GCTGAACGAG	AAACGTAAAA	TGATATAAAT	ATCAATATAT	TAAATTAGAT	TTTGCATAAA
181	AAACAGACTA	CATAATACTG	TAAAACACAA	CATATCCAGT	CACTATGAAT	CAACTACTTA
241	GATGGTATTA	GTGACCTGTA	GTCGACCGAC	AGCCTTCCAA	ATGTTCTTCG	GGTGATGCTG
301	CCAACCTTAG	CGACCGACAG	CCTTCCAAAT	GTTCTTCTCA	AACGGAATCG	TCGTATCCAG
361	CCTACTCGCT	ATTGTCCTCA	ATGCCGTATT	AAATCATAAA	AAGAAATAAG	AAAAAGAGGT
421	GCGAGCCTCT	TTTTTGTGTG	ACAAAATAAA	AACATCTACC	TATTCATATA	CGCTAGTGTC
481	ATAGTCCTGA	AAATCATCTG	CATCAAGAAC	AATTTACAAA	CTCTTATACT	TTTCTCTTAC
541	AAGTCGTTTC	GCTTCATCTG	GATTTTCAGC	CTCTATACTT	ACTAAACGTG	ATAAAGTTTC
601	TGTAATTCTT	ACTGTATCGA	CCTGCAGACT	GGCTGTGTAT	AAGGGAGCCT	GACATTTATA
661	TTCCCCAGAA	CATCAGGTGA	ATGGCGTTTT	TGATGTCATT	TTCGCGGTGG	CTGAGATCAG
721	CCACTTCTTC	CCCGATAACG	GAGACCGGCA	CACTGGCCAT	ATCGGTGGTC	ATCATGCGCC
781	AGCTTTTCATC	CCCGATATGC	ACCACCGGGT	AAAGTTTCACG	GGAGACTTTA	TCTGACAGCA
841	GACGTGCACT	GGCCAGGGGG	ATCACCATCC	GTCGCCCGGG	CGTGTCATAA	ATATCACTCT
901	GTACATCCAC	AAACAGACGA	TAACGGCTCT	CTCTTTTATA	GGTGTAACC	TTAAACTGCA
961	TTTCACCACT	CCCTGTTCTC	GTGAGCAAAA	GAGCCGTTCA	TTTCAATAAA	CCGGGCGACC
1021	TCAGCATATC	CTTCCTGATT	TTCCGCTTTT	CAGCGTTTCG	CACGCAGACG	ACGGGCTTCA
1081	TTCTGCATGG	TTGTGCTTAC	CAGACCGGAG	ATATTGACAT	CATATATGCC	TTGAGCAACT
1141	GATAGCTGTC	GCTGTCAACT	GTCACTGTAA	TACGCTGCTT	CATAGCACAC	CTCTTTTGA
1201	CATACTTCGG	GTATACATAT	CAGTATATAT	TCTTATACCG	CAAAAATCAG	CGCGCAAATA
1261	CGCATACTGT	TATCTGGCTT	TTAGTAAGCC	GGATCCACGC	GATTACGCCC	CGCCCTGCCA
1321	CTCATCGCAG	TACTGTTGTA	ATTCATTAAG	CATTCTGCCG	ACATGGAAGC	CATCACAGAC
1381	GGCATGATGA	ACCTGAATCG	CCAGCGGCAT	CAGCACCTTG	TCGCCTTGGC	TATAATATTT
1441	GCCCATGGTG	AAAACGGGGG	CGAAGAAGTT	GTCCATATTG	GCCACGTTTA	AATCAAAACT
1501	GGTGAAATC	ACCCAGGGAT	TGGCTGAGAC	GAAAAACATA	TTCTCAATAA	ACCCTTTAGG
1561	GAAATAGGCC	AGGTTTTTAC	CGTAACACGC	CACATCTTGC	GAATATATGT	GTAGAAACTG
1621	CCGGAATCG	TCGTGGTATT	CACTCCAGAG	CGATGAAAC	GTTTCAGTTT	GCTCATGGAA
1681	AACGGTGTA	CAAGGGTGAA	CACTATCCCA	TATCACCAGC	TCACCGTCTT	TCATTGCCAT
1741	ACGGAATTCC	GGATGAGCAT	TCATCAGGCG	GGCAAGAATG	TGAATAAAGG	CCGGATAAAA
1801	CTTGCTGCTT	TTTTTCTTTA	CGGTCTTTAA	AAAGGCCGTA	ATATCCAGCT	GAACGGTCTG
1861	GTTATAGGTA	CATTGAGCAA	CTGACTGAAA	TGCCTCAAAA	TGTTCTTTAC	GATGCCATTG
1921	GGATATATCA	ACGGTGATAT	ATCCAGTGAT	TTTTTCTCTC	ATTTAGCTT	CCTTAGCTCC
1981	TGAAAATCTC	GATAACTCAA	AAAATACGCC	CGGTAGTGAT	CTTATTTTAT	TATGGTGAAA
2041	GTTGGAACCT	CTTACGTGCC	GATCAACGTC	TCATTTTCGC	CAAAAGTTGG	CCCAGGGCTT
2101	CCCGGTATCA	ACAGGGACAC	CAGGATTTAT	TTATTCTGCG	AAGTGATCTT	CCGTCACAGG
2161	TATTTATTTC	GCGCAAAGTG	CGTCGGGTGA	TGCTGCCAAC	TTAGTCGACT	ACAGGTCACT
2221	AATACCATCT	AAGTAGTTGA	TTCATAGTGA	CTGGATATGT	TGTGTTTTAC	AGTATTATGT
2281	AGTCTGTTTT	TTATGCAAAA	TCTAATTTAA	TATATTGATA	TTTATATCAT	TTTACGTTTC
2341	TCGTTTCAGT	TTCTTGATCA	AAGTTGGCAT	TATAAGAAAG	CATTGCTTAT	CAATTTGTTG
2401	CAACGAACAG	GTCATATCA	GTCAAAATAA	AATCATTATT	TGCCATCCAG	CTGCAGCTCT
2461	GGCCCGTGTC	TCAAAATCTC	TGATGTTTACA	TTGCACAAGA	TAAAAATATA	TCATCATGAA
2521	CAATAAAACT	GTCTGCTTAC	ATAAACAGTA	ATACAAGGGG	TGTTATGAGC	CATATTCAAC
2581	GGGAAACGTC	GAGGCCGCGA	TTAAATTCCA	ACATGGATGC	TGATTTATAT	GGGTATAAAT
2641	GGGCTCGCGA	TAATGTCGGG	CAATCAGGTG	CGACAATCTA	TCGCTTGAT	GGGAAGCCCC
2701	ATGCGCCAGA	GTTGTTTCTG	AAACATGGCA	AAGGTAGCGT	TGCCAATGAT	GTTACAGATG

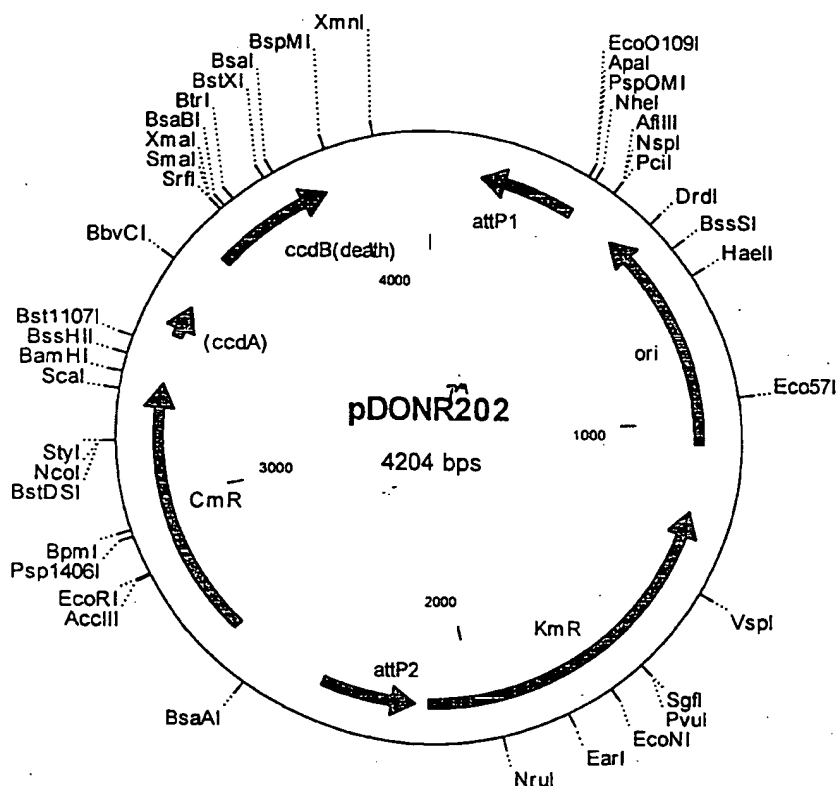
FIGURE 49B

146/240

2761 AGATGGTCAG ACTAAACTGG CTGACGGAAT TTATGCCTCT TCCGACCATC AAGCATTTTA
2821 TCCGTACTCC TGATGATGCA TGGTTACTCA CCACTGCGAT CCCC GGAAAA ACAGCATTCC
2881 AGGTATTAGA AGAATATCCT GATTCAAGTG AAAATATTGT TGATGCGCTG GCAGTGTTCC
2941 TGC GCCGGT GCATTCGATT CCTGTTTGTA ATTGTCCTTT TAACAGCGAT CGCGTATTTT
3001 GTCTCGCTCA GGC GCAATCA CGAATGAATA ACGGTTTGTT TGATGCGAGT GATTTTGATG
3061 ACGAGCGTAA TGGCTGGCCT GTTGAACAAG TCTGGAAAGA AATGCATAAA CTTT TGCCAT
3121 TCTCACC GGA TTCAGTCGTC ACTCATGGTG ATTTCTCACT TGATAACCTT ATTTT TGACG
3181 AGGGGAAATT AATAGGTTGT ATTGATGTTG GACGAGTCGG AATCGCAGAC CGATACCAGG
3241 ATCTTGCCAT CCTATGGAAC TGCCTCGGTG AGTTTTCTCC TTCATTACAG AAACGGCTTT
3301 TTCAAAAATA TGGTATTGAT AATCCTGATA TGAATAAATT GCAGTTTCAT TTGATGCTCG
3361 ATGAGTTTTT CTAATCAGAA TTGGTTAATT GGT TGTAACA CTGGCAGAGC ATTACGCTGA
3421 CTTGACGGGA CGGCGCAAGC TCATGACCAA AATCCCTTAA CGTGAGTTTT CGTTCCACTG
3481 AGCGTCAGAC CCCGTAGAAA AGATCAAAGG ATCTTCTTGA GATCCTTTTT TTCTGCGCGT
3541 AATCTGCTGC TTGCAAAACA AAAAACACC GCTACCAGCG GTGGTTTGTT TGCCGGATCA
3601 AGAGCTACCA ACTCTTTTTC CGAAGGTAAC TGGCTTCAGC AGAGCGCAGA TACCAAATAC
3661 TGTCTTCTA GTGTAGCCGT AGTTAGGCCA CCACTTCAAG AACTCTGTAG CACCGCCTAC
3721 ATACCTCGCT CTGCTAATCC GTTACCAGT GGCTGCTGCC AGTGGCGATA AGTCGTGTCT
3781 TACCGGGTTG GACTCAAGAC GATAGTTACC GGATAAGGCG CAGCGGTCGG GCTGAACGGG
3841 GGGTTCGTGC ACACAGCCCA GCTTGGAGCG AACGACCTAC ACCGAACTGA GATACCTACA
3901 GCGTGAGCTA TGAGAAAGCG CCACGCTTCC CGAAGGGAGA AAGGCGGACA GGTATCCGGT
3961 AAGCGGCAGG GTCGGAACAG GAGAGCGCAC GAGGGAGCTT CCAGGGGGAA ACGCCTGGTA
4021 TCTTTATAGT CCTGTGCGGT TTCGCCACCT CTGACTTGAG CGTCGATTTT TGTGATGCTC
4081 GTCAGGGGGG CGGAGCCTAT GGAAAAACGC CAGCAACGCG GCCTTTTTTAC GGTTCCTGGC
4141 CTTTTGCTGG CCTTTTGCTC ACATGTTCTT TCCTGCGTTA TCCCCTGATT CTGTGGATAA
4201 CCGTATTACC GCTAGCCAGG AAGAGTTTGT AGAAACGCAA AAAGGCCATC CGTCAGGATG
4261 GCCTTCTGCT TAGTTTGATG CCTGGCAGTT TATGGCGGGC GTCCTGCCCC CCACCTCCG
4321 GGCCGTTGCT TCACAACGTT CAAATCCGCT CCCGGCGGAT TTGTCCTACT CAGGAGAGCG
4381 TTCACCGACA AACAACAGAT AAAACGAAAG GCCCAGTCTT CCGACTGAGC CTTTCGTTTT
4441 ATTTGATGCC TGGCAGTTCC CTACTCTCGC

FIGURE 49C

147/240
FIGURE 50A: pDONR202 (kan^R)



pDONR202 4204 bp

<u>Location (Base Nos.)</u>			<u>Gene Encoded</u>		
369..127			attP1		
486..1059			ori		
1228..2107			KmR		
2381..2140			attP2		
2629..3288			CmR		
3408..3492			inactivated ccdA		
3630..3935			ccdB		
1	CGGCATTGAG	GACAATAGCG	AGTAGGCTGG	ATACGACGAT	TCCGTTTGAG
61	GGAAGGCTGT	CGGTCGACTA	AGTTGGCAGC	ATCACCCGAA	GAACATTTGG
121	GTCGACTACA	GGTCACTAAT	ACCATCTAAG	TAGTTGATTG	ATAGTGAATG
181	GTTTTACAGT	ATTATGTAGT	CTGTTTTTTA	TGCAAAATCT	AATTTAATAT
241	ATATCATTTT	ACGTTTCTCG	TTCAGCTTTT	TTGTACAAAG	TTGGCATTAT
301	TGCTCATCAA	TTTGTGTCAG	CGAACAGGTC	ACTATCAGTC	AAAATAAAAT
361	GGCCCCGAGAT	CCATGCTAGC	GGTAATACGG	TTATCCACAG	AATCAGGGGA
421	AAGAACATGT	GAGCAAAAGG	CCAGCAAAAG	GCCAGGAACC	GTAATAAAGG
481	GCGTTTTTCC	ATAGGCTCCG	CCCCCTGAC	GAGCATCACA	AAAATCGACG
541	AGGTGGCGAA	ACCCGACAGG	ACTATAAAGA	TACCAGGCGT	TTCCCCCTGG
601	GTGCGCTCTC	CTGTTCCGAC	CCTGCCGCTT	ACCGGATACC	TGTCCGCCTT
661	GGAAGCGTGG	CGCTTTCTCA	TAGCTCACGC	TGTAGGTATC	TCAGTTCGGT
721	CGCTCCAAGC	TGGGCTGTGT	GCACGAACCC	CCCGTTCAGC	CCGACCGCTG
781	GGTAACTATC	GTCTTGAGTC	CAACCCGGTA	AGACACGACT	TATCGCCACT
841	ACTGGTAACA	GGATTAGCAG	AGCGAGGTAT	GTAGGCGGTG	CTACAGAGTT
901	TGGCCTAACT	ACGGCTACAC	TAGAAGGACA	GTATTTGGTA	TCTGCGCTCT
961	GTTACCTTCG	GAAAAAGAGT	TGGTAGCTCT	TGATCCGGCA	AACAAACCAC
1021	GGTGGTTTTT	TTGTTTGCAA	GCAGCAGATT	ACGCGCAGAA	AAAAAGGATC
1081	CCTTTGATCT	TTTCTACGGG	GTCGTACGCT	CAGTGGAACG	AAAACCTCAG
1141	TTGGTCATGA	GCTTGCGCCG	TCCCGTCAAG	TCAGCGTAAT	GCTCTGCCAG
1201	AATTAACCAA	TTCTGATTAG	AAAACTCAT	CGAGCATCAA	ATGAAACTGC
1261	TATCAGGATT	ATCAATACCA	TATTTTGTAA	AAAGCCGTTT	CTGTAATGAA
1321	CACCGAGGCA	GTTCCATAGG	ATGGCAAGAT	CCTGGTATCG	GTCTGCGATT
1381	CAACATCAAT	ACAACCTATT	AATTTCCCCT	CGTCAAAAAT	AAGGTTATCA
1441	CACCATGAGT	GACGACTGAA	TCCGGTGAGA	ATGGCAAAAG	TTTATGCATT
1501	CTTGTTCAAC	AGGCCAGCCA	TTACGCTCGT	CATCAAAATC	ACTCGCATCA
1561	TATTCATTCT	TGATTGCGCC	TGAGCGAGAC	GAAATACGCG	ATCGCTGTTA
1621	TACAAACAGG	AATCGAATGC	AACCGCGCA	GGAACACTGC	CAGCGCATCA
1681	CACCTGAATC	AGGATATTCT	TCTAATACCT	GGAATGCTGT	TTTTCCGGGG
1741	TGAGTAACCA	TGCATCATCA	GGAGTACGGA	TAAAATGCTT	GATGGTCGGA
1801	ATCCCGTCAG	CCAGTTTAGT	CTGACCATCT	CATCTGTAAC	ATCATTGGCA
1861	TGCCATGTTT	CAGAAACAAC	TCTGCGCAT	CGGGCTTCCC	ATACAAGCGA
1921	CACCTGATTG	CCCGACATTA	TCGCGAGCCC	ATTTATACCC	ATATAAATCA
1981	TGGAATTTAA	TCGCGGCCTC	GACGTTTCCC	GTTGAATATG	GCTCATAACA
2041	TACTGTTTAT	GTAAGCAGAC	AGTTTATTG	TTTATGATGA	TATATTTTAA
2101	TGTAACATCA	GAGATTTTGA	GACACGGGCC	AGAGCTGCAG	CTGGATGGCA
2161	TTATTTTGAC	TGATAGTGAC	CTGTTGCTTG	CAACAAATTG	ATAAGCAATG
2221	ATGCCAACTT	TGTACAAGAA	AGCTGAACGA	GAAACGTAAA	ATGATATAAA
2281	TAAATTAGA	TTTTGCATAA	AAAACAGACT	ACATAATACT	GTAATAACAA
2341	TCACTATGAA	TCAACTACTT	AGATGGTATT	AGTGACCTGT	AGTCGACTAA
2401	TCACCCGACG	CACTTTGCGC	CGAATAAATA	CCTGTGACGG	AAGATCACTT
2461	ATAAATCCTG	GTGTCCCTGT	TGATACCGGG	AAGCCCTGGG	CCAACCTTTG
2521	GACGTTGATC	GGCACGTAA	AGGTTCCAAC	TTTCAACATA	ATGAAATAAG
2581	GGCGTATTTT	TTGAGTTATC	GAGATTTTCA	GGAGCTAAAG	AAGCTAAAT
2641	ATCACTGGAT	ATACCACCGT	TGATATATCC	CAATGGCATC	GTAAAGAACA
2701	TTTCAGTCAG	TTGCTCAATG	TACCTATAAC	CAGACCGTTC	AGCTGGATAT

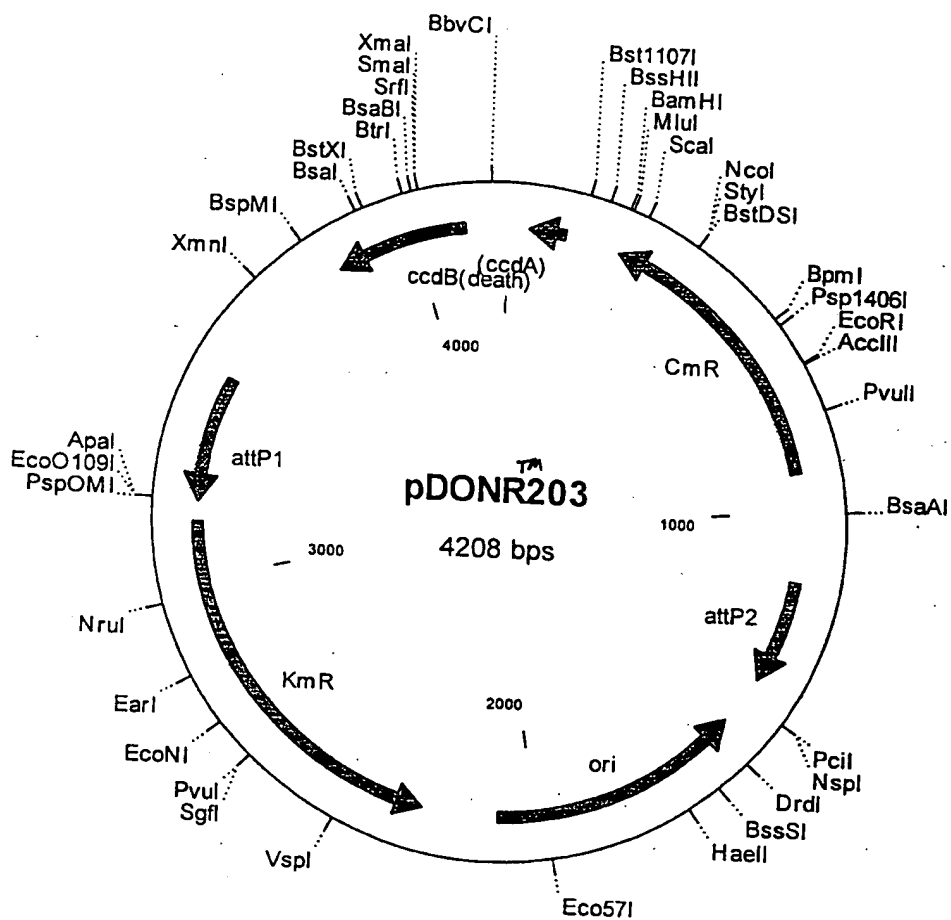
FIGURE 50B

149/240

2761 TTAAAGACCG TAAAGAAAAA TAAGCACAAG TTTTATCCGG CCTTTATTCA CATTCTTGCC
2821 CGCCTGATGA ATGCTCATCC GGAATTCCGT ATGGCAATGA AAGACGGTGA GCTGGTGATA
2881 TGGGATAGTG TTCACCCCTTG TTACACCGTT TTCCATGAGC AAACCTGAAAC GTTTTCATCG
2941 CTCTGGAGTG AATACCACGA CGATTTCCGG CAGTTTCTAC ACATATATTC GCAAGATGTG
3001 GCGTGTTACG GTGAAAACCT GGCCTATTTC CCTAAAGGGT TTATTGAGAA TATGTTTTTC
3061 GTCTCAGCCA ATCCCTGGGT GAGTTTCACC AGTTTTGATT TAAACGTGGC CAATATGGAC
3121 AACTTCTTCG CCCCCGTTTT CACCATGGGC AAATATTATA CGCAAGGCGA CAAGGTGCTG
3181 ATGCCGCTGG CGATTCAGGT TCATCATGCC GTCTGTGATG GCTTCCATGT CGGCAGAATG
3241 CTTAATGAAT TACAACAGTA CTGCGATGAG TGGCAGGGCG GGGCGTAATC GCGTGGATCC
3301 GGCTTACTAA AAGCCAGATA ACAGTATGCG TATTTGCGCG CTGATTTTTG CGGTATAAGA
3361 ATATATACTG ATATGTATAC CCGAAGTATG TCAAAAAGAG GTGTGCTATG AAGCAGCGTA
3421 TTACAGTGAC AGTTGACAGC GACAGCTATC AGTTGCTCAA GGCATATATG ATGTCAATAT
3481 CTCCGGTCTG GTAAGCACAA CCATGCAGAA TGAAGCCCGT CGTCTGCCGTG CCGAACGCTG
3541 GAAAGCGGAA AATCAGGAAG GGATGGCTGA GGTCGCCCCG TTTATTGAAA TGAACGGCTC
3601 TTTTGCTGAC GAGAACAGGG ACTGGTGAAA TGCAGTTTAA GGTTTACACC TATAAAGAG
3661 AGAGCCGTTA TCGTCTGTTT GTGGATGTAC AGAGTGATAT TATTGACACG CCGGGCGGAC
3721 GGATGGTGAT CCCCCTGGCC AGTGCACGTC TGCTGTCAGA TAAAGTCTCC CGTGAACTTT
3781 ACCCGGTGGT GCATATCGGG GATGAAAGCT GGCGCATGAT GACCACCGAT ATGGCCAGTG
3841 TGCCGGTCTC CGTTATCGGG GAAGAAGTGG CTGATCTCAG CCACCGCGAA AATGACATCA
3901 AAAACGCCAT TAACCTGATG TTCTGGGGAA TATAAATGTC AGGCTCCCTT ATACACAGCC
3961 AGTCTGCAGG TCGATACAGT AGAAATTACA GAAACTTTAT CACGTTTAGT AAGTATAGAG
4021 GCTGAAAATC CAGATGAAGC CGAACGACTT GTAAGAGAAA AGTATAAGAG TTGTGAAATT
4081 GTTCTTGATG CAGATGATTT TCAGGACTAT GACACTAGCG TATATGAATA GGTAGATGTT
4141 TTTATTTTGT CACACAAAAA AGAGGCTCGC ACCTCTTTTT CTTATTTCTT TTTATGATTT
4201 AATA

FIGURE 50C.

FIGURE 51A pDONR203 (kan^R)



151/240

pDONR203 4208 bp

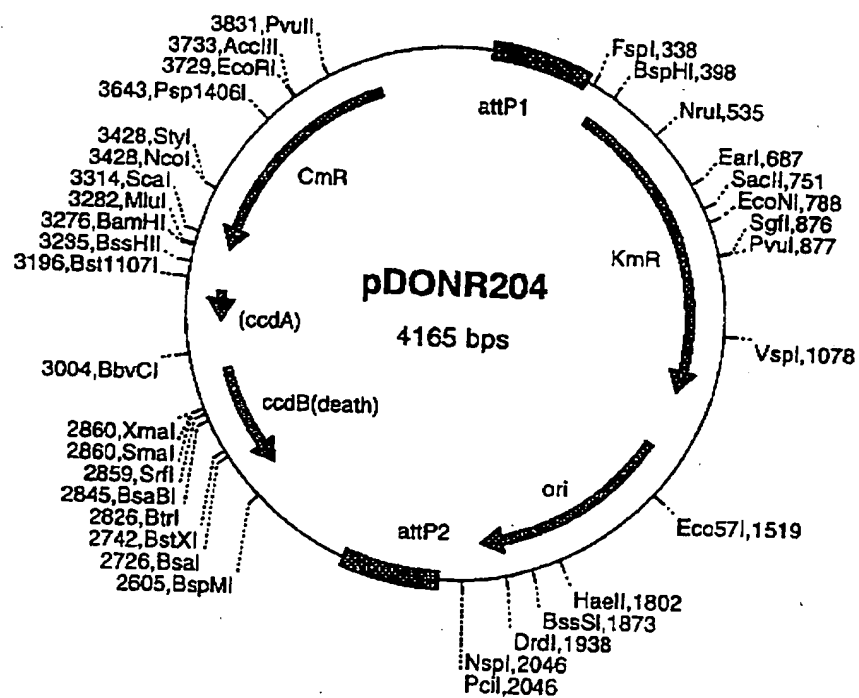
<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
47..131		inactivated ccdA
251..910		CmR
1158..1398		attP2
1509..2082		ori
2251..3130		KmR
3464..3174		attP1
3812..4117		ccdB
1	GCGTTCGGCA CGCAGACGAC GGGCTTCATT CTGCATGGTT GTGCTTACCA GACCGGAGAT	
61	ATTGACATCA TATATGCCTT GAGCAACTGA TAGCTGTGCG TGTCAACTGT CACTGTAATA	
121	CGCTGCTTCA TAGCACACCT CTTTTTGACA TACTTCGGGT ATACATATCA GTATATATTC	
181	TTATACCGCA AAAATCAGCG CGCAAATACG CATACTGTTA TCTGGCTTTT AGTAAGCCCG	
241	ATCCACGCGT TTACGCCCCG CCCTGCCACT CATCGCAGTA CTGTTGTAAT TCATTAAGCA	
301	TTCTGCCGAC ATGGAAGCCA TCACAGACGG CATGATGAAC CTGAATCGCC AGCGGCATCA	
361	GCACCTGTGC GCCTTGCGTA TAATATTTGC CCATGGTGAA AACGGGGGCG AAGAAGTTGT	
421	CCATATTGGC CACGTTTAAA TCAAACTGG TGAAACTCAC CCAGGGATTG GCTGAGACGA	
481	AAAACATATT CTCAATAAAC CCTTTAGGGA AATAGGCCAG GTTTTCACCG TAACACGCCA	
541	CATCTTGCGA ATATATGTGT AGAAACTGCC GGAAATCGTC GTGGTATTCA CTCCAGAGCG	
601	ATGAAAACGT TTCAGTTTGC TCATGGAAAA CGGTGTAACA AGGGTGAACA CTATCCCATA	
661	TCACCAGCTC ACCGTCTTTC ATTGCCATAC GGAATTCGG ATGAGCATTC ATCAGGCGGG	
721	CAAGAATGTG AATAAAGGCC GGATAAACT TGTGCTTATT TTTCTTTACG GTCTTTAAAA	
781	AGGCCGTAAT ATCCAGCTGA ACGGTCTGGT TATAGGTACA TTGAGCAACT GACTGAAATG	
841	CCTCAAAATG TTCTTTACGA TGCCATTGGG ATATATCAAC GGTGGTATAT CCAGTGATTT	
901	TTTTCTCCAT TTTAGCTTCC TTAGCTCCTG AAAATCTCGA TAACTCAAAA AATACGCCCC	
961	GTAGTGATCT TATTTCAATTA TGGTGAAAGT TGGAACCTCT TACGTGCCGA TCAACGTCTC	
1021	ATTTTCGCCA AAGTTGGGCC CAGGGCTTCC CGGTATCAAC AGGGACACCA GGATTTATTT	
1081	ATTCTGCGAA GTGATCTTCC GTCACAGGTA TTTATTCGGC GCAAAGTGCG TCGGGTGATG	
1141	CTGCCAACTT AGTCGACTAC AGGTCACTAA TACCATCTAA GTAGTTGATT CATAGTGACT	
1201	GGATATGTTG TGTTTTACAG TATTATGTAG TCTGTTTTTT ATGCAAAATC TAATTTAATA	
1261	TATTGATATT TATATCATTT TACGTTTCTC GTTCAGCTTT CTTGTACAAA GTTGGCATT	
1321	TAAGAAAGCA TTGCTTATCA ATTTGTTGCA ACGAACAGGT CACTATCAGT CAAAATAAAA	
1381	TCATTATTG CCATCCAGCT AGCGGTAATA CGGTTATCCA CAGAATCAGG GGATAACGCA	
1441	GGAAAGAACA TGTGAGCAAA AGGCCAGCAA AAGGCCAGGA ACCGTAAAAA GGCCGCGTTG	
1501	CTGGCGTTTT TCCATAGGCT CCGCCCCCTC GACGAGCATC ACAAATTCG ACGCTCAAGT	
1561	CAGAGGTGGC GAAACCCGAC AGGACTATAA AGATAACAGG CGTTTCCCCC TGGAAAGCTCC	
1621	CTCGTGCGCT CTCCTGTTCC GACCCTGCCG CTTACCGGAT ACCTGTCCGC CTTTCTCCCT	
1681	TCGGGAAGCG TGGCGCTTTC TCATAGCTCA CGCTGTAGGT ATCTCAGTTC GGTGTAGGTC	
1741	GTTGCTCCA AGCTGGGCTG TGTGCACGAA CCCCCGTTT AGCCCGACCG CTGCGCCTTA	
1801	TCCGGTAACT ATCGTCTTGA GTCCAACCCG GTAAGACACG ACTTATCGCC ACTGGCAGCA	
1861	GCCACTGGTA ACAGGATTAG CAGAGCGAGG TATGTAGGCG GTGCTACAGA GTTCTTGAAG	
1921	TGGTGGCCTA ACTACGGCTA CACTAGAAGA ACAGTATTTG GTATCTGCGC TCTGCTGAAG	
1981	CCAGTTACCT TCGGAAAAAG AGTTGGTAGC TCTTGATCCG GCAAACAAAC CACCGCTGGT	
2041	AGCGGTGGTT TTTTTGTTG CAAGCAGCAG ATTACGCGCA GAAAAAAGG ATCTCAAGAA	
2101	GATCCTTTGA TCTTTTCTAC GGGGTCTGAC GCTCAGTGGA ACGAAAACTC ACGTTAAGGG	
2161	ATTTTGGTCA TGAGCTTGCG CCGTCCCGTC AAGTCAGCGT AATGCTCTGC CAGTGTTACA	
2221	ACCAATTAAC CAATTCTGAT TAGAAAACT CATCGAGCAT CAAATGAAAC TGCAATTTAT	
2281	TCATATCAGG ATTATCAATA CCATATTTTT GAAAAAGCCG TTTCTGTAAT GAAGGAGAAA	
2341	ACTCACCGAG GCAGTTCCAT AGGATGGCAA GATCCTGGTA TCGGTCTGCG ATTCCGACTC	
2401	GTCCAACATC AATACAACCT ATTAATTTCC CCTCGTCAAA AATAAGGTTA TCAAGTGAGA	
2461	AATCACCATG AGTGACGACT GAATCCGGTG AGAATGGCAA AAGTTTATGC ATTTCTTTCC	
2521	AGACTTGTTC AACAGGCCAG CCATTACGCT CGTCATCAAA ATCACTCGCA TCAACCAAAC	
2581	CCTTATTTCAT TCGTGATTGC GCCTGAGCGA GACGAAATAC GCGATCGCTG TTAAGAGGAC	
2641	AATTACAAAC AGGAATCGAA TGCAACCGGC GCAGGAACAC TGCCAGCGCA TCAACAATAT	
2701	TTTCACCTGA ATCAGGATAT TCTTCTAATA CCTGGAATGC TGTTTTTCCG GGGATCGCAG-	

FIGURE 51B

2761 TGGTGAGTAA CCATGCATCA TCAGGAGTAC GGATAAAATG CTTGATGGTC GGAAGAGGCA
2821 TAAATTCCGT CAGCCAGTTT AGTCTGACCA TCTCATCTGT AACATCATTG GCAACGCTAC
2881 CTTTGCCATG TTTCAGAAAC AACTCTGGCG CATCGGGCTT CCCATACAAG CGATAGATTG
2941 TCGCACCTGA TTGCCCCGACA TTATCGCGAG CCCATTTATA CCCATATAAA TCAGCATCCA
3001 TGTTGGAATT TAATCGCGGC CTCGACGTTT CCCGTTGAAT ATGGCTCATA ACACCCCTTG
3061 TATTACTGTT TATGTAAGCA GACAGTTTTA TTGTTTATGA TGATATATTT TTATCTTGTG
3121 CAATGTAACA TCAGAGATTT TGAGACACGG GCCAGAGCTG CAGCTAGCAT GGATCTCGGG
3181 CCCCATAATA TGATTTTATT TTGACTGATA GTGACCTGTT CGTTGCAACA AATTGATGAG
3241 CAATGCTTTT TTATAATGCC AACTTTGTAC AAAAAAGCTG AACGAGAAAC GTAAAATGAT
3301 ATAAATATCA ATATATTAAA TTAGATTTTG CATAAAAAAC AGACTACATA ATACTGTAAA
3361 ACACAACATA TCCAGTCACT ATGAATCAAC TACTTAGATG GTATTAGTGA CCTGTAGTCG
3421 ACCGACAGCC TTCCAAATGT TCTTCGGGTG ATGCTGCCAA CTTAGTCGAC CGACAGCCTT
3481 CCAAATGTTT TTCTCAAACG GAATCGTCGT ATCCAGCCTA CTCGCTATTG TCCTCAATGC
3541 CGTATTAAAT CATAAAAAGA AATAAGAAAA AGAGGTGCGA GCCTCTTTTT TGTGTGACAA
3601 AATAAAAAACA TCTACCTATT CATATACGCT AGTGTCATAG TCCTGAAAAAT CATCTGCATC
3661 AAGAACAATT TCACAACTCT TATACTTTTC TCTTACAAGT CGTTCCGGCTT CATCTGGATT
3721 TTCAGCCTCT ATACTTACTA AACGTGATAA AGTTTCTGTA ATTTCTACTG TATCGACCTG
3781 CAGACTGGCT GTGTATAAGG GAGCCTGACA TTTATATTCC CCAGAACATC AGGTTAATGG
3841 CGTTTTTTGAT GTCATTTTCG CGGTGGCTGA GATCAGCCAC TTCTTCCCGG ATAACGGAGA
3901 CCGGCACACT GGCCATATCG GTGGTCATCA TCGCCAGCT TTCATCCCCG ATATGCACCA
3961 CCGGGTAAAG TTCACGGGAG ACTTTATCTG ACAGCAGACG TGCACTGGCC AGGGGGATCA
4021 CCATCCGTCG CCCGGGCGTG TCAATAATAT CACTCTGTAC ATCCACAAAC AGACGATAAC
4081 GGCTCTCTCT TTTATAGGTG TAAACCTTAA ACTGCATTTT ACCAGTCCCT GTTCTCGTCA
4141 GCAAAGAGC CGTTCATTTT AATAAACCGG GCGACCTCAG CCATCCCTTC CTGATTTTCC
4201 GCTTTCCA

FIGURE 51C

FIGURE 52A pDONR204 (kanR)



154/240

pDONR204 4165 bp

```
1 CGGCATTGAG GACAATAGCG AGTAGGCTGG ATACGACGAT TCCGTTTGAG AAGAACATTT
61 GGAAGGCTGT CGGTCGACTA CAGGTCACATA ATACCATCTA AGTAGTTGAA TCATAGTGAC
121 TGGATATGTT GTGTTTTTACA GTATTATGTA GTCTGTTTTT TATGCAAAAT CTAATTTAAT
181 ATATTGATAT TTATATCATT TTACGTTTCT CGTTCAGCTT TTTTGTACAA AGTTGGCATT
241 ATAAAAAAGC ATTGCTTATC AATTTGTTGC AACGAACAGG TCACTATCAG TCAAAATAAA
301 ATCATTATTT GGGGCCCGAG ATCCATGCTA GCTGCAGTGC GCAGGGCCCG TGTCTCAAAA
361 TCTCTGATGT TACATTGCAC AAGATAAAAA TATATCATCA TGAACAATAA AACTGTCTGC
421 TTACATAAAC AGTAATACAA GGGGTGTTAT GAGCCATATT CAACGGGAAA CGTCTTGCTG
481 GAGGCCCGCA TTAAATTCCA ACATGGATGC TGATTTATAT GGGTATAAAT GGGCTCGCGA
541 TAATGTCGGG CAATCAGGTG CGACAATCTT TCGATTGTAT GGAAGCCCG ATGCGCCAGA
601 GTTGTTCCTG AAACATGGCA AAGGTAGCGT TGCCAATGAT GTTACAGATG AGATGGTCAG
661 ACTAACTGG CTGACGGAAT TTATGCCTCT TCCGACCATC AAGCATTTTA TCCGTACTCC
721 TGATGATGCA TGGTTACTCA CCACTGCGAT CCGCGGGAAA ACAGCATTCC AGGTATTAGA
781 AGAATATCCT GATTCAGGTG AAAATATTGT TGATGCGCTG GCAGTGTTC TGCGCCGGTT
841 GCATTGATCT CCTGTTTGTA ATTGTCCTTT TAACAGCGAT CGCGTATTTC GTCTCGCTCA
901 GCGCAATCA CGAATGAATA ACGTTTGGT TGATGCGAGT GATTTTGATG ACGAGCGTAA
961 TGGCTGGCCT GTTGAACAAG TCTGGAAAGA AATGCATACG CTTTGGCCAT TCTCACCGBA
1021 TTCAGTCGTC ACTCATGGTG ATTTCTCACT TGATAACCTT ATTTTGTACG AGGGGAAATT
1081 AATAGGTTGT ATTGATGTTG GACGAGTCGG AATCGCAGAC GATACCCAGG ATCTTGCCAT
1141 CCTATGGAAC TGCCTCGGTG AGTTTTCTCC TTCATTACAG AAACGGCTTT TTCAAAAATA
1201 TGGTATTGAT AATCCTGATA TGAATAAATT GCAGTTTCAT TTGATGCTCG ATGAGTTTTT
1261 CTAATCAGAA TTGGTTAATT GGTGTGAACA CTGGCAGAGC ATTACGCTGA CTTGACGGGA
1321 CCGCGNCATG ACCAAAATCC CTTAACGTGA GTTTTCGTTT CACTGAGCGT CAGACCCCGT
1381 AGAAAAGATC AAAGGATCTT CTTGAGATCC TTTTTTCTG CGCGTAATCT GCTGCTTGCA
1441 AACAAAAAAA CCACCCTAC CAGCGGTGGT TTGTTTGCCG GATCAAGAGC TACCAACTCT
1501 TTTTCCGAAG GTAACGGCTT TCAGCAGAGC GCAGATACCA AATACTGTCC TTCTAGTGTA
1561 GCCGTAGTTA GGCCACCCT TCAAGAACTC TGTAGCACCG CCTACATACC TCGCTCTGCT
1621 AATCCTGTTA CCAGTGGCTG CTGCCAGTGG CGATAAGTCG TGTCTTACCG GGTGGACTC
1681 AAGACGATAG TTACCGGATA AGGCGCAGCG GTCGGGCTGA ACGGGGGGTT CGTGACACACA
1741 GCCCAGCTTG GAGCGAACGA CCTACACCGA ACTGAGATAC CTACAGCGTG AGCTATGAGA
1801 AAGCGCCACG CTTCCCGAAG GGAGAAAGGC GGACAGGTAT CCGGTAAGCG GCAGGGTCGG
1861 AACAGGAGAG CGCACGAGGG AGCTTCCAGG GGGAAACGCC TGGTATCTTT ATAGTCTGT
1921 CGGGTTTCGC CACCTCTGAC TTGAGCGTCG ATTTTGTGTA TGCTCGTCAG GGGGGCGGAG
1981 CCTATGGAAG AACGCCAGCA ACGCGGCTTT TTTACGGTTC CTGGCCTTTT GCTGGCCTTT
2041 TGCTCACATG TTCTTTCTCG CGTTATCCCC TGATTCTGTG GATAACCGTA TTACCGTAG
2101 CTGGATCGGC AAATAATGAT TTTATTTTGA CTGATAGTGA CCTGTTCTGT GCAACAAATT
2161 GATAAGCAAT GCTTTTTTAT AATGCCAAT TTGTACAAGA AAGCTGAACG AGAAACGTAA
2221 AATGATATAA ATATCAATAT ATTAAATTAG ATTTTGCATA AAAACAGAC TACATAATAC
2281 TGTAAACAC AACATATCCA GTCACATAGA TTCAACTACT TAGATGGTAT TAGTGACCTG
2341 TAGTCGACTA AGTTGGCAGC ATCACCAGAC GCACCTTTCG CCGAATAAAT ACCTGTGACG
2401 GAAGATCACT TCGCAGAATA AATAAATCCT GGTGTCCCTG TTGATACCGG GAAGCCCTGG
2461 GCCAATCTTT GCGGAAAATG AGACGTTGAT CGGCACATTT CACAATCTT ATACTTTTCT
2521 CTTACAAGTC GTTCGGCTTC ATCTGGATTT TCAGCCTCTA TACTTACTAA ACGTGATAAA
2581 GTTCTGTAA TTTCTACTGT ATCGACCTGC AGACTGGCTG TGTATAACGG AGCCTGACAT
2641 TTATATTCCC CAGAACATCA GGTTAATGGC GTTTTTGATG TCATTTTCGC GGTGGCTGAG
2701 ATCAGCCACT TCTTCCCGCA TAACGGAGAC CGGCACACTG GCCATATCGG TGGTCATCAT
2761 GCGCCAGCTT TCATCCCGCA TATGACCAC CCGGTAAAGT TCACGGGAGA CTTTATCTGA
2821 CAGCAGACGT GCACTGGCCA GGGGGATCAC CATCCGTCGC CCGGGCGTGT CAATAATATC
2881 ACTCTGTACA TCCACAAACA GACGATAACG GCTCTCTCTT TTATAGGTGT AAACCTTAAA
2941 CTGCAATTTCA CCAGTCCCTG TTCTCGTCAG CAAAAGAGCC GTTCATTTCA ATAAACCGGG
3001 CGACCTCAGC CATCCCTTCC TGATTTTCCG CTTTCCAGCG TTCGGCACGC AGACGACGGG
3061 CTTCAATCTG CATGGTTGTG CTTACCAGAC CGGAGATATT GACATCATAT ATGCTTGAG
3121 CAACTGATAG CTGTCGCTGT CAACTGTCAC TGTAATACGC TGCTTCATAG CACACCTCTT-
```

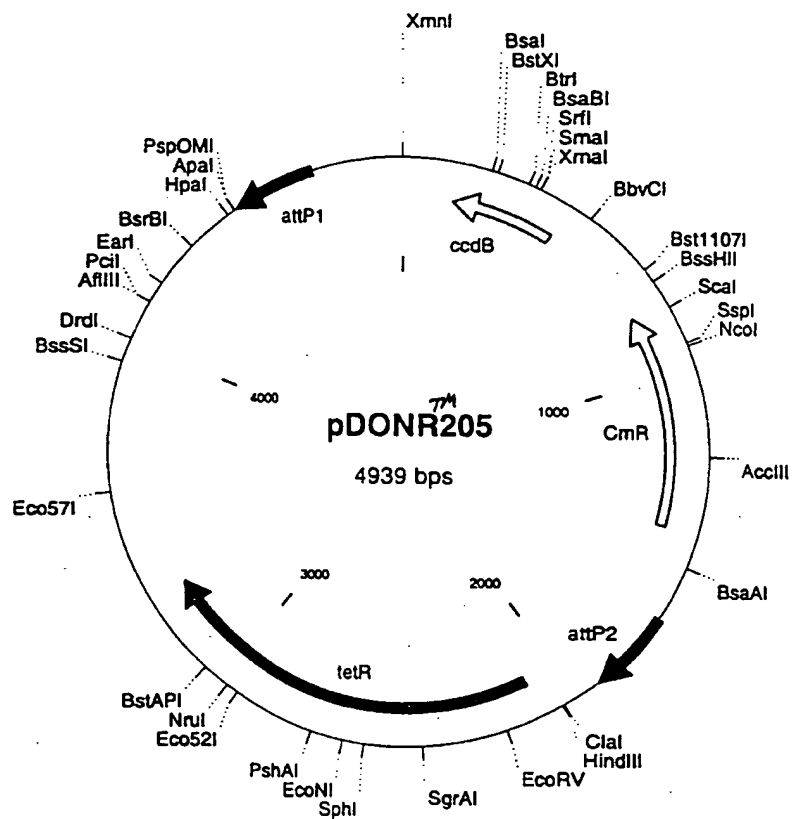
FIGURE 52B

3181 TTTGACATAC TTCGGGTATA CATATCAGTA TATATTCTTA TACCGCAAAA ATCAGCGCGC
3241 AAATACGCAT ACTGTTATCT GGCTTTTAGT AAGCCGGATC CACGCGTTTA CGCCCCGCCC
3301 TGCCACTCAT CGCAGTACTG TTGTAATTCA TTAAGCATTG TGCCGACATG GAAGCCATCA
3361 CAGACGGCAT GATGAACCTG AATCGCCAGC GGCATCAGCA CCTTGTCGCC TTGCGTATAA
3421 TATTTGCCCA TGGTGAAAAC GGGGGCGAAG AAGTTGTCCA TATTGGCCAC GTTTAAATCA
3481 AAACCTGGTGA AACTCACCCA GGGATTGGCT GAGACGAAAA ACATATTCTC AATAAACCCCT
3541 TTAGGGAAAT AGGCCAGGTT TTCACCGTAA CACGCCACAT CTTGCGAATA TATGTGTAGA
3601 AACTGCCGGA AATCGTCGTG GTATTCACTC CAGAGCGATG AAAACGTTTC AGTTTGCTCA
3661 TGGAAAACGG TGTAACAAGG GTGAACACTA TCCCATATCA CCAGCTCACC GTCTTTCATT
3721 GCCATACGGA ATTCCGGATG AGCATTATC AGGCGGGCAA GAATGTGAAT AAAGGCCGGA
3781 TAAACTTGT GCTTATTTTT CTTTACGGTC TTTAAAAAGG CCGTAATATC CAGCTGAACG
3841 GTCTGGTTAT AGGTACATTG AGCAACTGAC TGAAATGCCT CAAAATGTTC TTTACGATGC
3901 CATTGGGATA TATCAACGGT GGTATATCCA GTGATTTTTT TCTCCATTTT AGCTTCCTTA
3961 GCTCCTGAAA ATCTCGATAA CTCAAAAAAT ACGCCCGGTA GTGATCTTAT TTCATTATGG
4021 TGAAAGTTGG AACCTCTTAC TGTTCTTGAT GCAGATGATT TTCAGGACTA TGACACTAGC
4081 ATATATGAAT AGGTAGATGT TTTTATTTTG TCACACAAAA AAGAGGCTCG CACCTCTTTT
4141 TCTTATTTCT TTTTATGATT TAATA

FIGURE 52C

156/240

Figure 53A: pDONR205 (tetR)



157/240

pDONR205 4939 bp

GGCATCAGCACCTTGTGCGCTTGCCTATAATATTTGCCCATGGTGAAAACGGGGGCGAAG
AAGTTGTCCATATTGGCCACGTTTAAATCAAACTGGTGAACTCACCAGGGATTGGCT
GAGACGAAAAACATATTCTCAATAAACCCCTTTAGGGAAATAGGCCAGGTTTTACCCGTAA
CACGCCACATCTTGC GAATATATGTGTAGAACTGCCGGAATCGTCGTGGTATTCACTC
CAGAGCGATGAAAACGTTTCAGTTTGCTCATGGAACCGGTGTAACAAGGGTGAACACTA
TCCCATATCACCAGCTCACCCTCTTTCAATTGCCATACGGAATCCGGATGAGCATTATC
AGGCGGGCAAGAAATGTGAATAAAGGCCGGATAAACTTGTGCTTATTTTCTTTACGGTC
TTTAAAAAGGCCGTAATATCCAGCTGAACGGTCTGGTTATAGGTACATTGAGCAACTGAC
TGAAATGCCTCAAAATGTTCTTTACGATGCCATTGGGATATATCAACGGTGGTATATCCA
GTGATTTTTTTCTCCATTTTAGCTTCCTTAGCTCCTGAAAATCTCGATAACTCAAAAAAT
ACGCCCCGGTAGTGATCTTATTTTCAATTATGGTGAAAGTTGGAACCTCTTACGTGCCGATCA
ACGTCTCATTTTTCGCCAAAAGTTGGCCCAGGGCTTCCCGGTATCAACAGGGACACCAGGA
TTTATTATTCTGCGAAGTGATCTTCCGTCACAGGTATTTATTTCGGCGCAAAGTGCCTCG
GGTGATGCTGCCAATTAGTCGACTACAGGTCACTAATACCATCTAAGTAGTTGATTGAT
AGTGACTGGATATGTTGTGTTTTACAGTATTATGTAGTCTGTTTTTTATGCAAAATCTAA
TTTAATATATTGATATTTATATCATTTTACGTTTCTCGTTTCACTTTCTTGTACAAAGTT
GGCATTATAAGAAAGCATTGCTTATCAATTGTTGCAACGAACAGGTCACTATCAGTCAA
AATAAAATCATTATTTGCCATCCAGCTGCAGCTCTGGCCCCGTGTCTCAAAATCTCTGATG
TTACATTGCACAAGATAAAAAATATATCATCATGAATTCTCATGTTTGACAGCTTATCATC
GATAAGCTTTAATGCGGTAGTTTATCACAGTTAAATTGCTAACGCAGTCAGGCACCGTGT
ATGAAATCTAACAATGCGCTCATCGTCATCCTCGGCACCGTCACCCTGGATGCTGTAGGC
ATAGGCTTGGTTATGCCGGTACTGCCGGGCTCTTGCGGGATATCGTCCATTCCGACAGC
ATCGCCAGTCACTATGGCGTGCTAGCGCTATATGCGTTGATGCAATTTCTATGCGCA
CCCGTTCTCGGAGCACTGTCCGACCGCTTTGGCCGCGCCAGTCCTGCTCGCTTCGCTA
CTTGGAGCCACTATCGACTACGCGATCATGGCGACCACACCCGTCCTGTGGATCCTCTAC
GCCGAGCGCATCGTGCCCGGCATCACCAGCGCCACAGGTGCGGTTGCTGGCGCCTATATC
GCCGACATCACCAGTGGGAAGATCGGGCTCGCCACTTCGGGCTCATGAGCGCTTGTTTC
GGCGTGGGTATGGTGGCAGGCCCCGTGGCCGGGGGACTGTTGGGCGCCATCTCCTTGCA
GCACCATTCCTTGCGGCGGCGGTGCTCAACGGCCTCAACCTACTACTGGGCTGCTTCCTA
ATGCAGGAGTCGCATAAGGGAGAGCGTCGACCGATGCCCTTGAGAGCCTTCAACCCAGTC
AGCTCCTTCCGGTGGGCGCGGGGCATGACTATCGTCGCCGCACTTATGACTGTCTCTTT
ATCATGCAACTCGTAGGACAGGTGCCGGCAGCGCTCTGGGTCATTTTTCGGCGAGGACCGC
TTTCGCTGGAGCGCGACGATGATCGGCTGTGCTTGGCGTATTCGGAATCTTGCACGCC
CTCGCTCAAGCCTTCGTCACTGCTCCCGCCACCAACGTTTCGGCGAGAAGCAGGCCATT
ATCGCCGGCATGGCGGCCGACGCGCTGGGCTACGTCTTGCTGGCGTTTCGCGACGCGAGGC
TGGATGGCCTTCCCCATTATGATTCTTCTCGCTTCCGGCGGCATCGGGATGCCCGCGTTG
CAGGCCATGCTGTCCAGGCAGGTAGATGACGACCATCAGGGACAGCTTCAAGGATCGCTC
GCGGCTCTTACCAGCCTAATCTCGATCATTGGACCGCTGATCGTCACGCGGATTTATGCC
GCCTCGGCGAGCACATGGAACGGGTGGCATGGATTGTAGGCGCCGCCCTATACCTTGTC
TGCTTCCCCGCTTGCGTCGCGTGTCATGGAGCCGGGCCACCTCGACCTGAATGGAAGCC
GGCGGCACCTCGCTAACGGATTCAACCACTCCAAGAATTGGAGCCAATCAATTCTTGCGGA
GAACTGTGAATGCGCAAAACCAACCCCTTGGCAGAATATCCATCGCATGACCAAAATCCC
TTAAGCTGAGTTTTCTGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTC
TTGAGATCCTTTTTTCTGCGCTAATCTGCTGCTTGCAAAACAAAAAACACCGCTACC
AGCGGTGGTTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTCCGAAGGTAAGTGGCTT
CAGCAGAGCGCAGATACCAATACTGTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTT
CAAGAACTCTGTAGCACCGCTACATACCTCGCTCTGCTAATCCTGTTACAGTGGCTGC
TGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAA
GGCGCAGCGGTGCGGCTGAACGGGGGTTCTGTCACACAGCCAGCTTGGAGCGAACGAC
CTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGG
GAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGA
GCTTCCAGGGGGAACGCCTGGTATCTTTATAGTCTGTGCGGTTTCGCCACCTCTGACT
TGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGCGGAGCCTATGAAAAACGCCAGCAA-

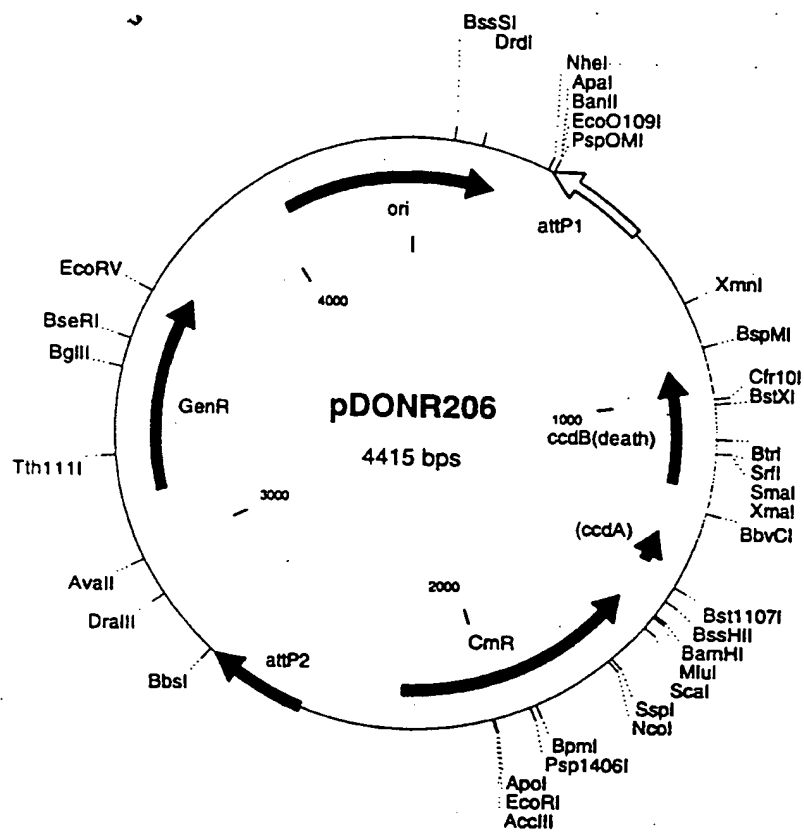
FIGURE 53B

158/240

CGCGGCCTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGC
GTTATCCCTGATTCTGTGGATAACCGTATTACCGCTAGCCAGGAAGAGTTGTAGAAAC
GCAAAAAGGCCATCCGTGAGGATGGCCTTCTGCTTAGTTTGATGCCTGGCAGTTTATGGC
GGGCGTCTGCCCCGCCACCTCCGGGCCGTTGCTTCACAACGTTCAAATCCGCTCCCCGGC
GGATTTGTCTACTCAGGAGAGCGTTCACCGACAAACAACAGATAAAACGAAAGGCCAG
TCTTCCGACTGAGCCTTTCGTTTTATTGATGCCTGGCAGTTCCTACTCTCGCGTTAAC
GCTAGCATGGATCTCGGGCCCCAAATAATGATTTTATTTTGACTGATAGTGACCTGTTG
TTGCAACAAATTGATGAGCAATGCTTTTTTATAATGCCAACTTGTACAAAAAGCTGAA
CGAGAAACGTAAATGATATAAATATCAATATATTAAATTAGATTTTGCATAAAAAACAG
ACTACATAATACTGTAAAACACAACATATCCAGTCACTATGAATCAACTACTTAGATGGT
ATTAGTGACCTGTAGTCGACCGACAGCCTTCCAAATGTTCTTCGGGTGATGCTGCCAACT
TAGTCGACCGACAGCCTTCCAAATGTTCTTCTCAAACGGAATCGTCGTATCCAGCCTACT
CGCTATTGTCTCAATGCCGTATTAAATCATAAAAAGAAATAAGAAAAAGAGGTGCGAGC
CTCTTTTTTGTGTGACAAAATAAAACATCTACCTATTCATATACGCTAGTGTCATAGTC
CTGAAAATCATCTGCATCAAGAACAATTTCACAACTCTTATACTTTTCTCTTACAAGTCG
TTCGGCTTCATCTGGATTTTCAGCCTCTATACTTACTAAACGTGATAAAGTTTCTGTAAT
TTCTACTGTATCGACCTGCGAGCTGGCTGTGTATAAGGGAGCCTGACATTTATATCCCC
AGAACATCAGGTTAATGGCGTTTTTGATGTCATTTTCGCGGTGGCTGAGATCAGCCACTT
CTTCCCCGATAACGGAGACCGGCACACTGGCCATATCGGTGGTCATCATGCGCCAGCTTT
CATCCCCGATATGCACCACCGGTTAAAGTTACGGGAGACTTTATCTGACAGCAGACGTG
CACTGGCCAGGGGATCACCATCCGTGCGCCGGGCGTGTCAATAATATCACTCTGTACAT
CCACAAACAGACGATAACGGCTCTCTCTTTTATAGGTGTAAACCTTAACTGCATTTAC
CAGTCCCTGTTCTCGTCAGCAAAAGAGCCGTTCAATTCATAAACCGGGCGACCTCAGCC
ATCCCTTCCTGATTTTCCGCTTTCCAGCGTTCGGCACGCAGACGACGGGCTTATTCTGC
ATGGTTGTGCTTACCAGACCGGAGATATTGACATCATATATGCCTTGAGCAACTGATAGC
TGTCGCTGTCAACTGTCACTGTAATACGCTGCTTCATAGCACACCTCTTTTGTACATACT
TCGGGTATACATATCAGTATATATTCTTATACCGCAAAAATCAGCGCGCAAAATACGCATA
CTGTTATCTGGCTTTTAGTAAGCCGGATCCACGCGATTACGCCCCGCCCTGCCACTCATC
GCAGTACTGTTGTAATTCATTAAGCATTCTGCCGACATGGAAGCCATCACAGACGGCATG
ATGAACCTGAATCGCCAGC

Figure 53C

159/240



160/240

pDONR206 4415 bp

CGGCATTGAGGACAATAGCGAGTAGGCTGGATACGACGATTCCGTTTGAGAAGAACATTT
GGAAGGCTGTGCGTCTGACTACAGGTCACTAATACCATCTAAGTAGTTGAATCATAGTGAC
TGGATATGTTGTGTTTTACAGTATTATGTAGTCTGTTTTTTATGCAAAATCTAATTTAAT
ATATTGATATTTATATCATTTTTACGTTTCTCGTTTCTGTTTGTACAAAGTTGGCATT
ATAAAAAAGCATTGCTTATCAATTTGTTGCAACGAACAGGTCACTATCAGTCAAAATAAA
ATCATTATTTGGGGCCCGAGATCCATGCTAGCGGTAATACGTTTATCCACAGAATCAGGG
GATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAG
GCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGA
CGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCT
GGAAGCTCCCTCGTGCCTCTCCTGTTCCGACCCCTGCCGTTACCGGATACCTGTCCGCG
TTTTCTCCCTTCGGGAAGCGTGGCGTTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCCG
GTGTAGGTCGTTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTACGCCCCGACCGC
TGCGCCTTATCCGGTAACATATCGTCTTGAGTCCAACCCGTAAGACACGACTTATCGCCA
CTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAG
TTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCT
CTGCTGAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAAACAAACC
ACCGCTGGTAGCGGTGGTTTTTTTGTGTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGA
TCTCAAGAAGATCCTTTGATCTTTCTACGGGGTCTGACGCTCAGTGGAAACGAAACTCA
CGTTAAGGGATTTTGGTTCATGNCGCCGTCCTCAAGTCAGCGTAATGCTCTGCCAGTGT
TACAACCAATTAACCAATTCTGATTAGAAAACTCATCGAGCATCAAATGAACTGCAAT
TTATTCATATCAGGATTATCAATACCATATTTTGAAGGCGCTTCTGTAATGAAGGA
GAAACTCACCGAGCAGTTCCATAGGATGGCAAGATCCTGGTATCGGTCTGCGATTCCG
ACTCGTCCAACATCAATACAACCTATTAGCCGAGGTCTTCCGATCTCCTGAAGCCAGGGC
AGATCCGTGCACAGCACCTTGCCGTAGAAGAAGCAGCAAGGCCGCAATGCCTGACGATGC
GTGGAGACCGAAACCTTGCGCTCGTTCGCCAGCCAGGACAGAAATGCCTCGACTTCGCTG
CTGCCCAAGGTTGCGGGTGACGCACACCGTGGAACCGGATGAAGGCACGAACCCAGTTG
ACATAAGCCTGTTTCGGTTTCGTAACTGTAATGCAAGTAGCGTATGCGCTCACGCAACTGG
TCCAGAACCTTGACCGAACGCAGCGGTGGTAACGGCGCAGTGGCGGTTTTTCATGGCTTGT
TATGACTGTTTTTTGTACAGTCTATGCCTCGGGCATCCAAGCAGCAAGCGCGTTACGCC
GTGGGTGATGTTTGTATGTTATGGAGCAGCAACGATGTTACGCAGCAGCAACGATGTTAC
GCAGCAGGGCAGTCGCCCTAAACAAAGTTAGGTGGCTCAAGTATGGGCATCATTGCGAC
ATGTAGGCTCGGCCCTGACCAAGTCAAATCCATGCGGGCTGCTCTTGATCTTTTCGGTTCG
TGAGTTCGGAGACGTAGCCACCTACTCCCAACATCAGCCGGACTCCGATTACCTCGGGAA
CTTGCTCCGTAGTAAGACATTCATCGCGCTTGCTGCCTTCGACCAAGAAGCGGTTGTTGG
CGCTCTCGCGGCTTACGTTCTGCCAGGTTTGAGCAGCCGCGTAGTGAGATCTATATCTA
TGATCTCGCAGTCTCCGGCGAGCACCGGAGGCAGGGCATTGCCACCGCGCTCATCAATCT
CCTCAAGCATGAGGCCAACGCGCTTGGTGCTTATGTGATCTACGTGCAAGCAGATTACGG
TGACGATCCCGCAGTGGCTCTCTATACAAAGTTGGGCATACGGGAAGAAGTGATGCATT
TGATATCGACCCAAGTACCGCCACCTAACAAATTCGTTCAAGCCGAGATCGGCTTCCCGGC
CTAATTTCCCCTCGTCAAAAATAAGGTTATCAAGTGAGAAATCACCATGAGTGACGACTG
AATCCGGTGAGAATGGCAAAAGCGTATGCATTTCTTCCAGACTTGTTCAACAGGCCAGC
CATTACGCTCGTCATCAAAATCACTCGCATCAACCAACCGTTATTTCATTCTGTGATTGCG
CCTGAGCGAGACGAAATACGCGATCGCTGTTAAAAGGACAATTACAAACAGGAATCGAAT
GCAACCGCGCAGGAACACTGCCAGCGCATCAACAATATTTTACCTGAATCAGGATATT
CTTCTAATACCTGGAATGCTGTTTTCCCGCGGATCGCAGTGGTGAGTAACCATGCATCAT
CAGGAGTACGGATAAAATGCTTGATGGTTCGGAAGAGGCATAAATCCGTCAGCCAGTTTA
GTCTGACCATCTCATCTGTAACATCATTGGCAACGCTACCTTTGCCATGTTTCAGAAACA
ACTCTGGCGCATCGGGCTTCCCATACAATCGAAAGATTGTGCGACCTGATTGCCCCGACAT
TATCGCGAGCCCATTTATACCCATATAAATCAGCATCCATGTTGGAATTTAATCGCGGCC
TCCAGCAAGACGTTTTCCGTTGAATATGGCTCATAAACCCCTTGTATTACTGTTTATGT
AAGCAGACAGTTTTATTGTTTCATGATGATATATTTTATCTTGTGCAATGTAACATCAGA
GATTTTGAGACACGGGCCNCGCGCACTGCAGCTGGATCGGCAAAATAATGATTTTATTTTG
ACTGATAGTGACCTGTTTCGTTGCAACAAATTGATAAGCAATGCTTTTTTATAATGCCAAC -

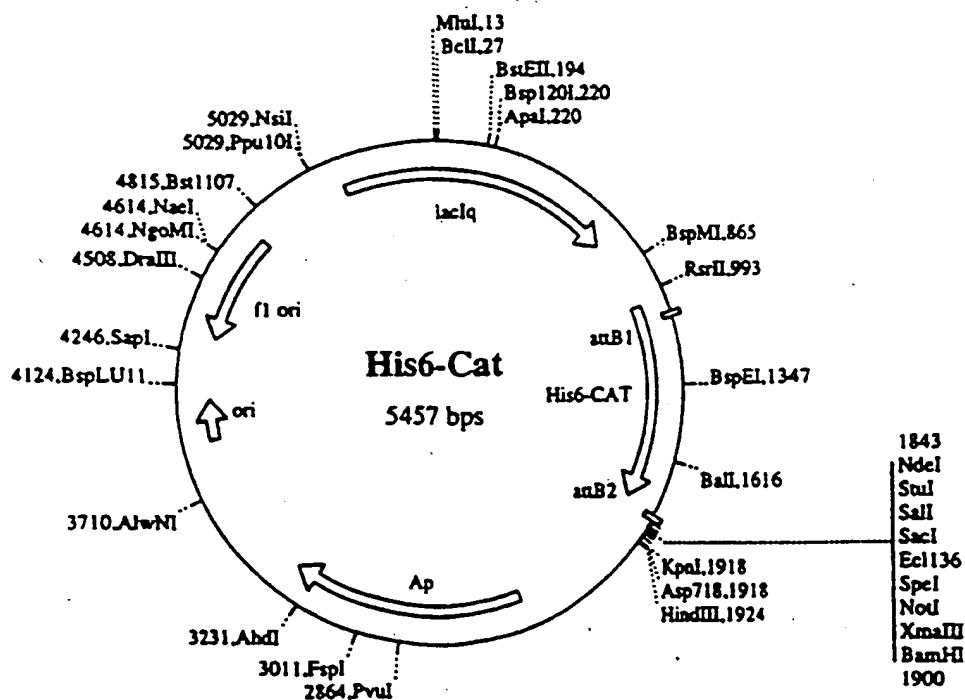
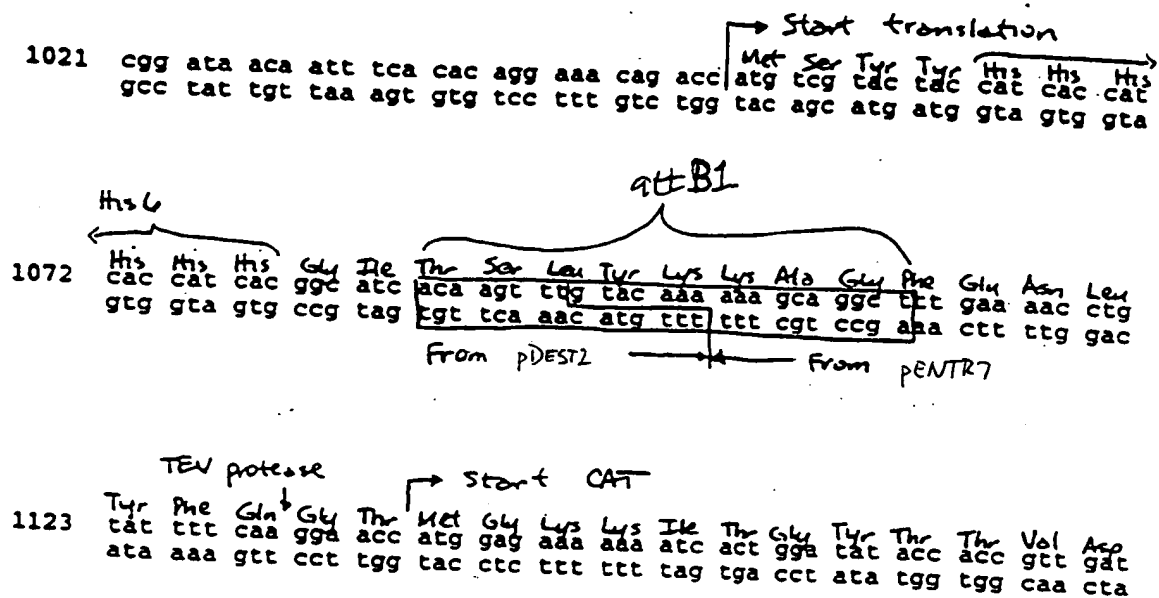
FIGURE 54B

161/240

TTTGTACAAGAAAGCTGAACGAGAAACGTAAAATGATATAAATATCAATATATTAAATTA
GATTTTGCATAAAAAACAGACTACATAAATACTGTAAAACACAACATATCCAGTCACTATG
ATTCAACTACTTAGATGGTATTAGTGACCTGTAGTCGACTAAGTTGGCAGCATCACCCGA
CGCACTTTGCGCCGAATAAATACCTGTGACGGAAGATCACTTCGCAGAATAAATAAATCC
TGGTGTCCCTGTTGATACCGGGAAGCCCTGGGCCAACTTTTGGCGAAAATGAGACGTTGA
TCGGCACGTAAGAGGTTCCAACCTTTACCATAATGAAATAAGATCACTACCGGGCGTATT
TTTTGAGTTATCGAGATTTTCAGGAGCTAAGGAAGCTAAAATGGAGAAAAAATCACTGG
ATATACCACCGTTGATATATCCCAATGGCATCGTAAAGAACATTTTGAGGCATTTTCAGTC
AGTTGCTCAATGTACCTATAACCAGACCGTTTCAGCTGGATATTACGGCCTTTTAAAGAC
CGTAAAGAAAAATAAGCACAAGTTTTATCCGGCCTTTATTACATTCTTGCCCGCCTGAT
GAATGCTCATCCGGAATTCGATGGAATGAAAGACGGTGAGCTGGTGATATGGGATAG
TGTTACCCCTGTTTACACCGTTTTCCATGAGCAAACTGAAACGTTTTTCATCGCTCTGGAG
TGAATACCACGACGATTTCCGGCAGTTTCTACACATATATTTCGAAGATGTGGCGTGTGA
CGGTGAAAACCTGGCCTATTTCCCTAAAGGGTTTATTGAGAAATATGTTTTTCGTCTCAGC
CAATCCCTGGGTGAGTTTACCAGTTTTGATTTAAACGTGGCCAATATGGACAACCTTCTT
CGCCCCGTTTTTACCATGGGCAATATTATACGCAAGGCGACAAGGTGCTGATGCCGCT
GGCGATTAGGTTTCATCATGCCGTCTGTGATGGCTTCCATGTGGCAGAATGCTTAATGA
ATTACAACAGTACTGCGATGAGTGGCAGGGCGGGCGTAAACCGTGGATCCGGCTTACT
AAAAGCCAGATAACAGTATGCGTATTTGCGCGCTGATTTTTGCGGTATAAGAATATATAC
TGATATGTATACCCGAAGTATGTCAAAAAGAGGTGTGCTATGAAGCAGCGTATTACAGTG
ACAGTTGACAGCGACAGCTATCAGTTGCTCAAGGCATATATGATGTCAATATCTCCGGTC
TGGTAAGCACAACCATGCAGAATGAAGCCCGTCTGCTGCGTGCCGAACGCTGGAAAGCGG
AAAATCAGGAAGGGATGGCTGAGGTGCGCCGGTTTATTGAAATGAACGGCTCTTTTGCTG
ACGAGAACAGGGACTGGTGAAATGCAGTTTAAAGGTTTACACCTATAAAAGAGAGAGCCGT
TATCGTCTGTTTGTGGATGTACAGAGTGATATTATTGACACGCCCCGGCGACGGATGGTG
ATCCCCCTGGCCAGTGACGCTCTGCTGTGATGATGACCAACGATATGGCCAGTGTGCCGGTC
GTGCATATCGGGGATGAAAGCTGGCGCATGATGACCACCGATATGGCCAGTGTGCCGGTC
TCCGTTATCGGGGAAGAAGTGGCTGATCTCAGCCACCGCGAAAATGACATCAAAAACGCC
ATTAACCTGATGTTCTGGGGAATATAAATGTCAGGCTCCGTTATACACAGCCAGTCTGCA
GGTCGATACAGTAGAAATTACAGAACTTTATCACGTTTGTAGTAAGTATAGAGGCTGAAAA
TCCAGATGAAGCCGAACGACTTGTAAAGAGAAAAGTATAAGAGTTGTGAAATTGTTCTTGA
TGCAGATGATTTTCAGGACTATGACACTAGCATATATGAATAGGTAGATGTTTTATTTT
GTCACACAAAAAAGAGGCTCGCACCTCTTTTCTTATTTCTTTTATGATTTAATA

FIGURE 54 C

Figure 55 An Entry (pENTR7) Clone of CAT Subcloned into pDEST2



163/240

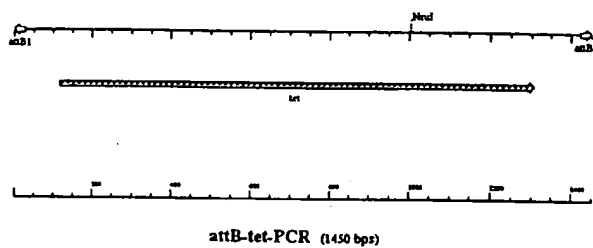
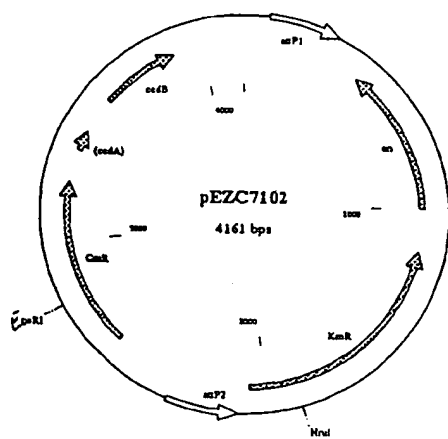


FIGURE 5b

164/240

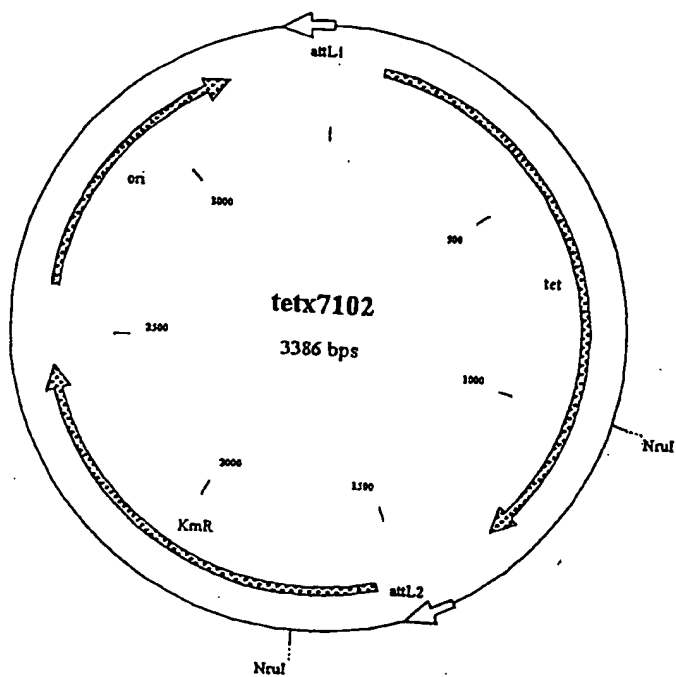


FIGURE 57

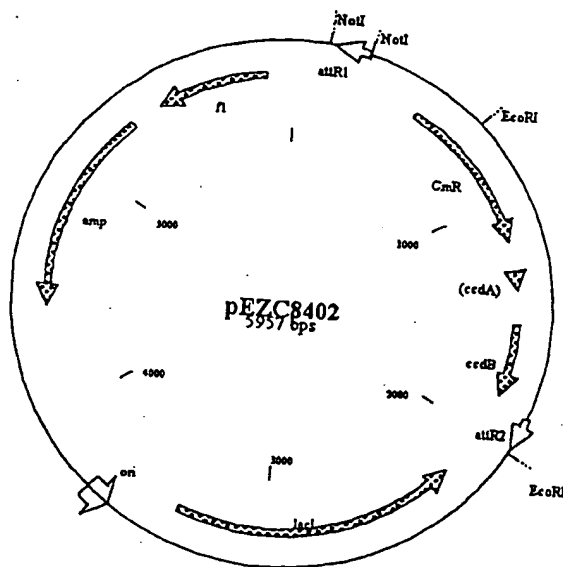


FIGURE 5B

166/240

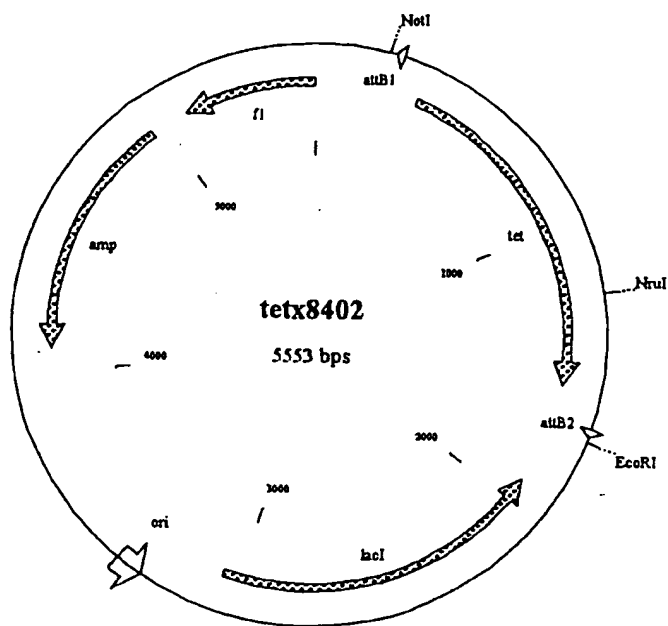


FIGURE 59

167/240

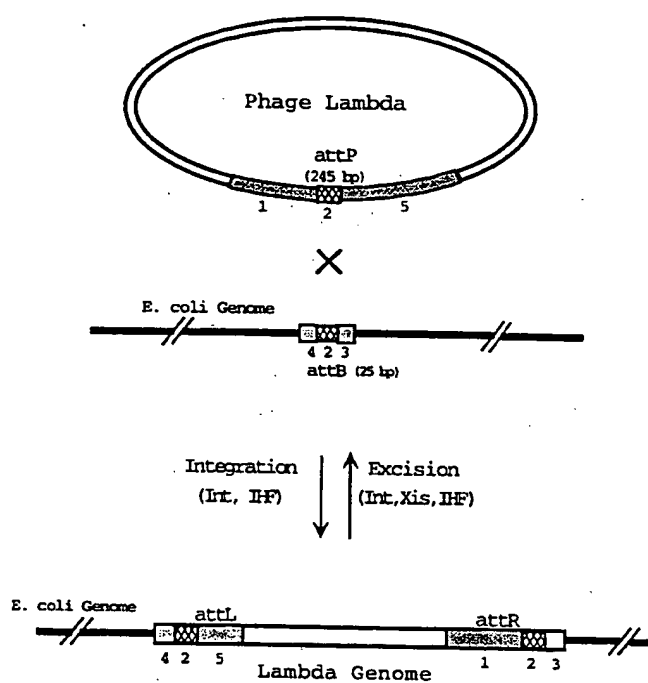


FIGURE 60

168/240

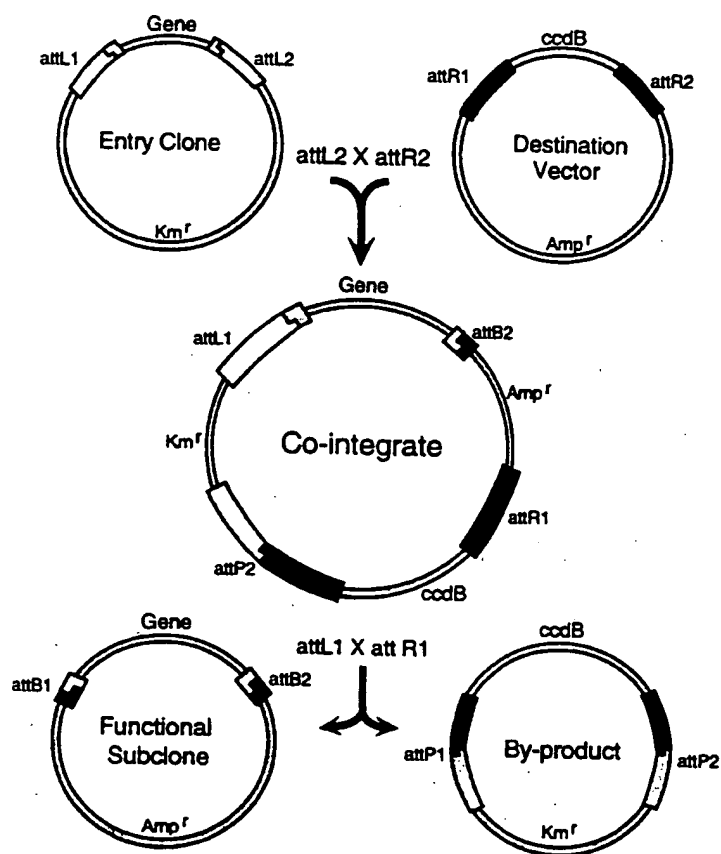


FIGURE 61

169/240

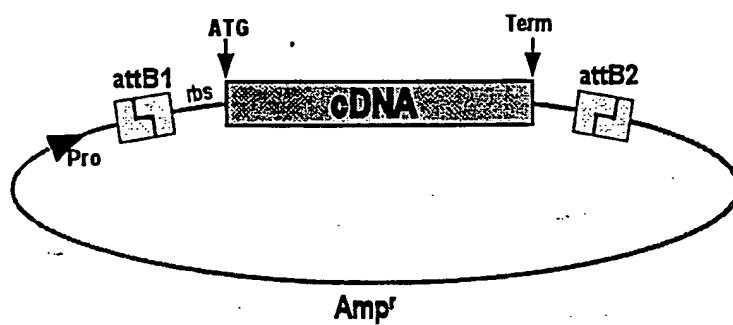
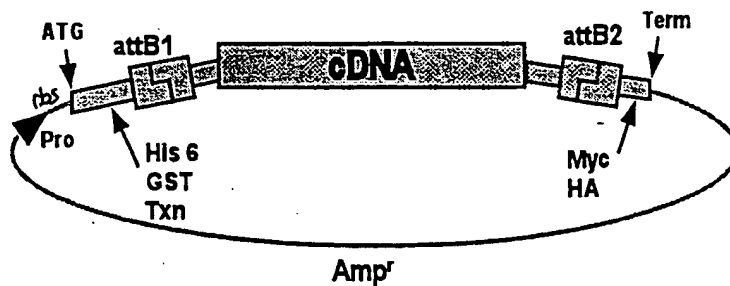
Native Protein Expression:**Fusion Protein Expression:**

FIGURE 62

170/240

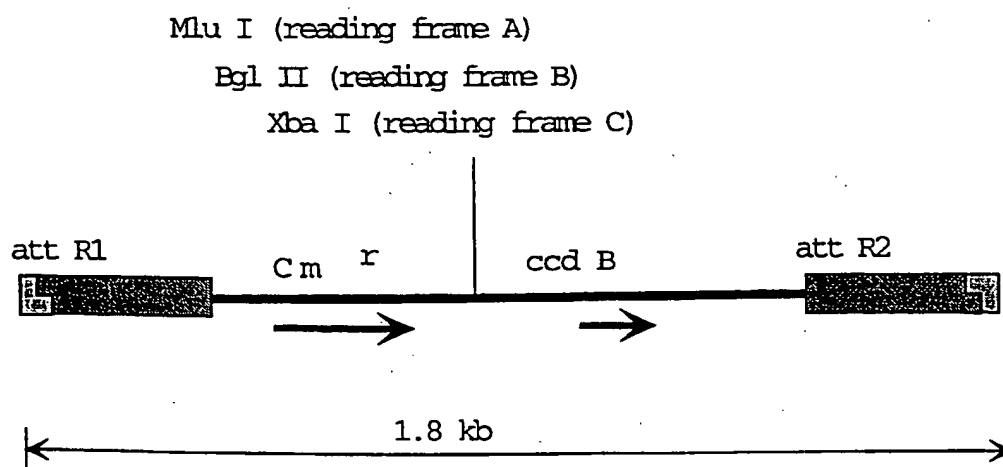


FIGURE 63

FIGURE 64A

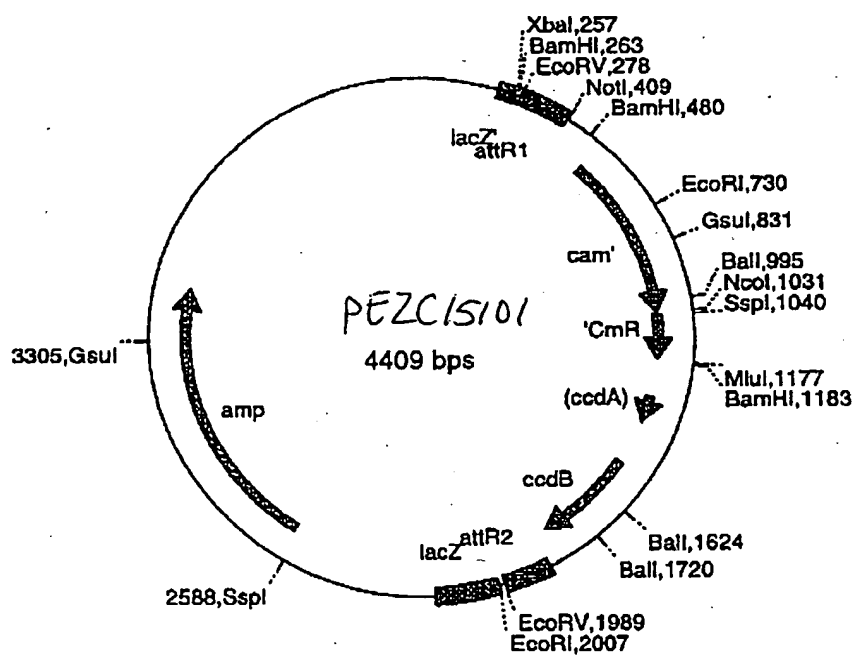
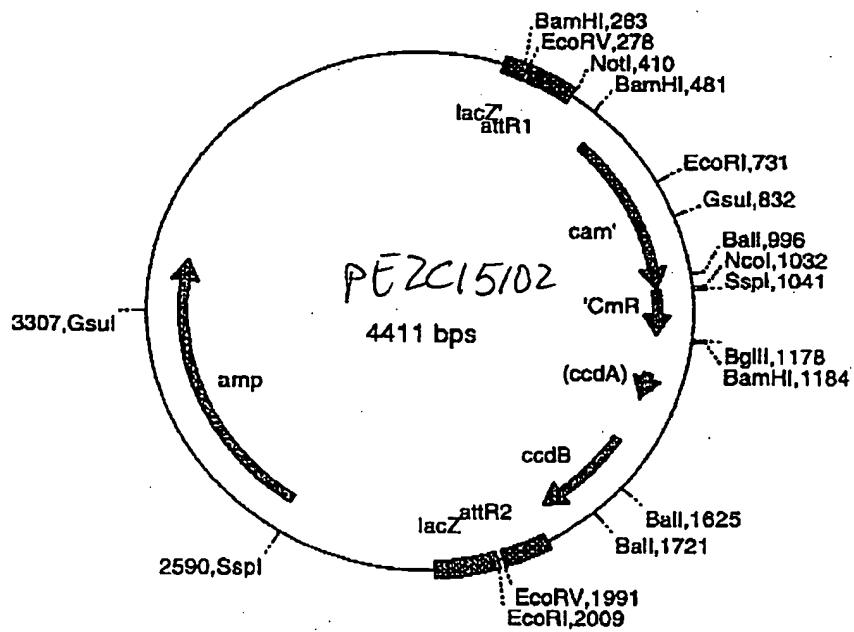
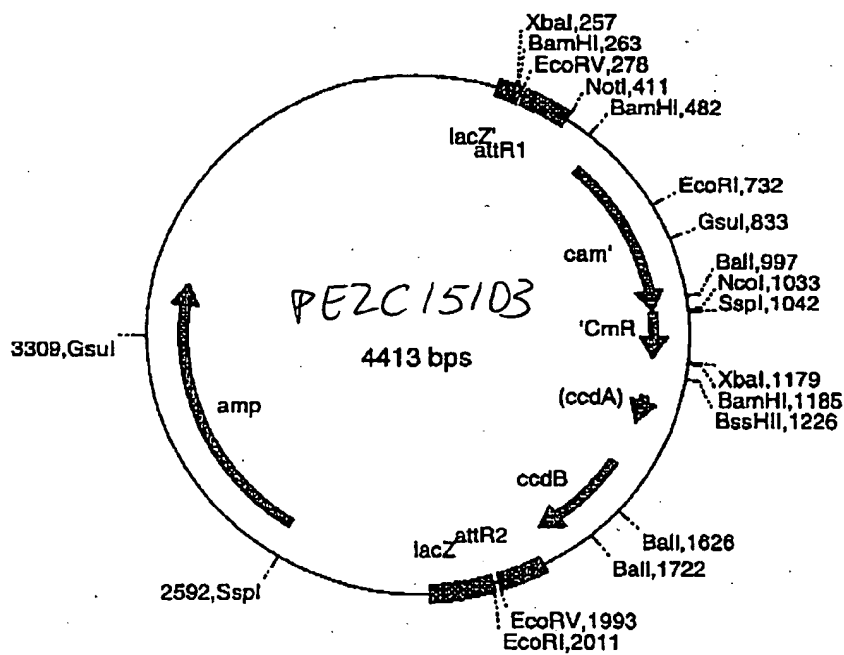


FIGURE 4aB



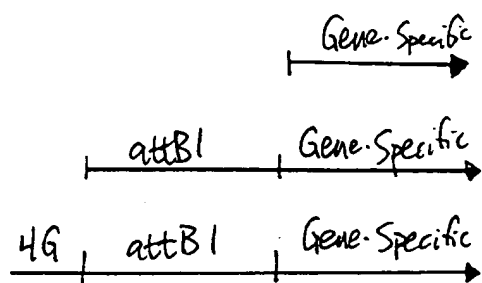
173/240

FIGURE 64C



Primers for Amplifying *tetR* and *ampR* for Cloning by Recombination

Primers



Reverse Primers

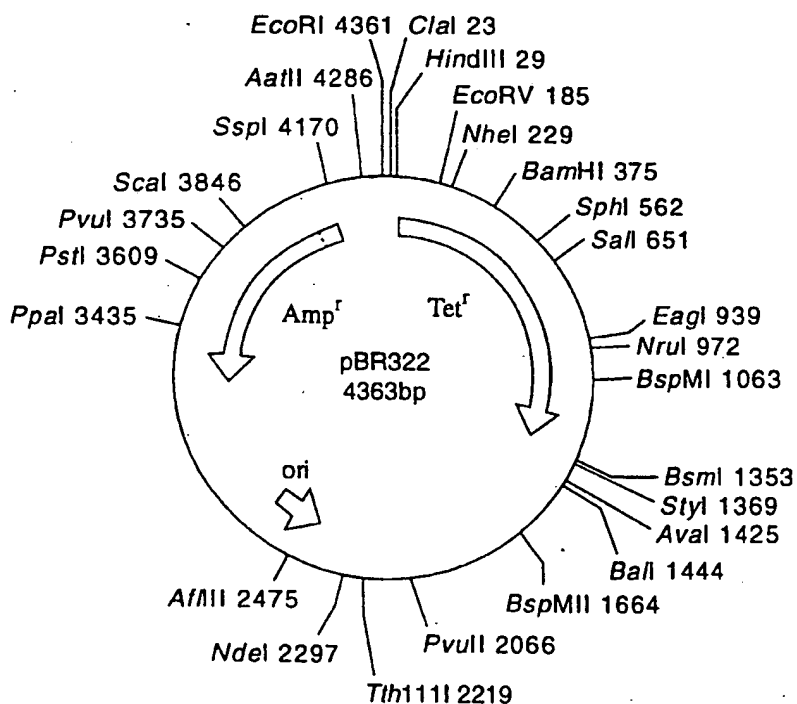
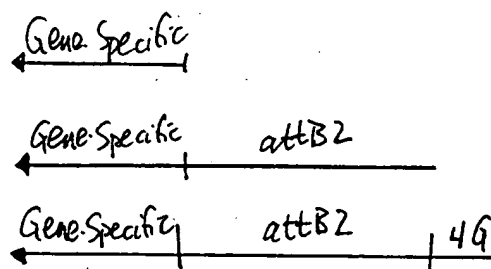


FIGURE 65

175/240

**Results of Cloning
tet and amp PCR Products
by Recombination**

PCR Product Used in GCS Reactions	No. Colonies Obtained (100 ul plated)	Form of DNA Analyzed	Colonies Obtained of Predicted Size
tet	6, 10	SC	0 of 8
attB-tet	9, 6	SC	1 of 8
attB+4G-tet	824, 1064	SC AvaI+Bam	7 of 7 7 of 7
amp	7, 13	SC	0 of 8
attB-amp	18, 22	SC	3 of 8
attB+4G-amp	3020, 3540	SC PstI	8 of 8 8 of 8
attB Plasmid (Pos. Control)	320, 394		

FIGURE 66

176/260

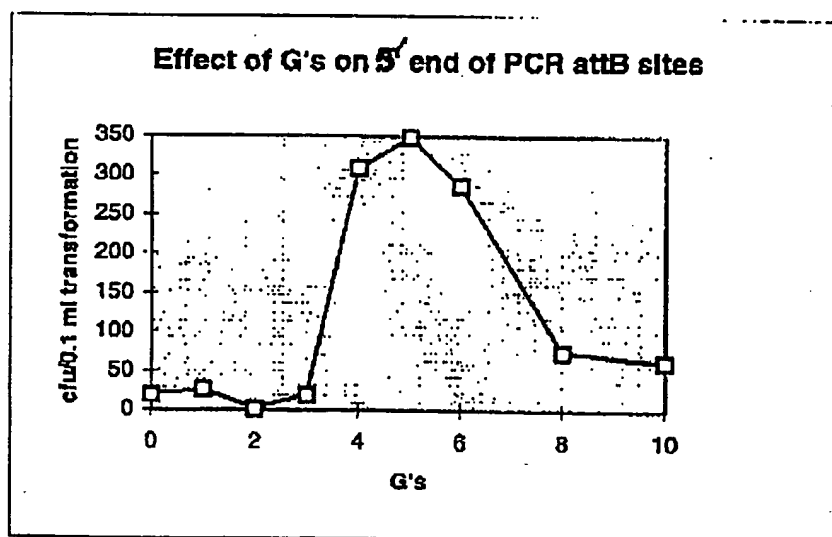


FIGURE 67

177/260

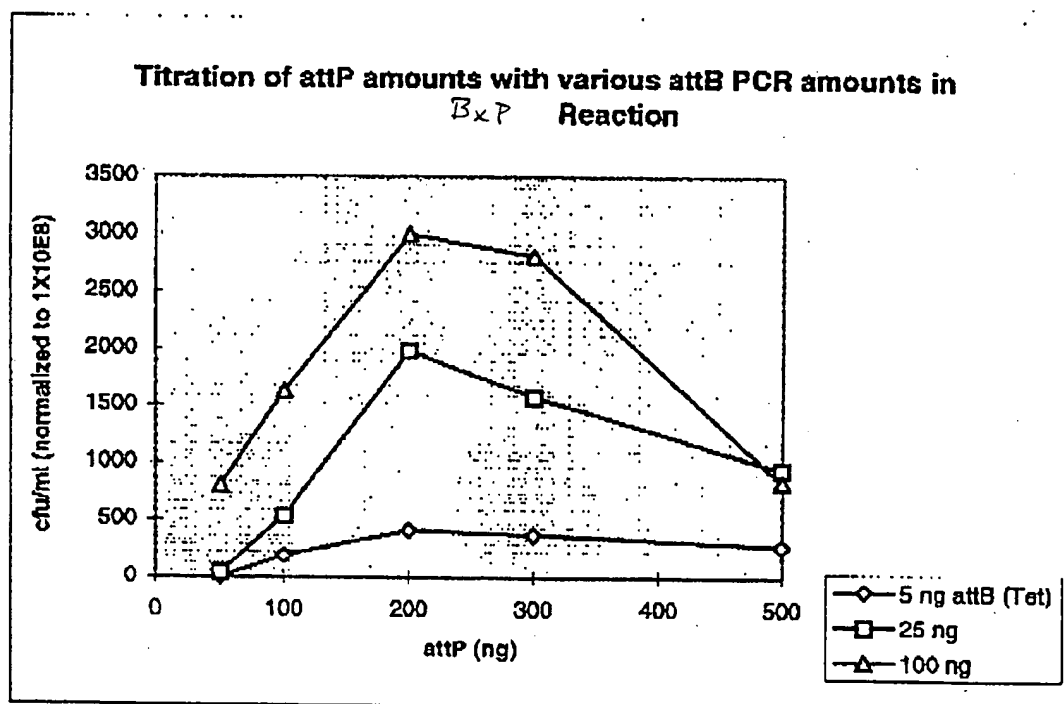
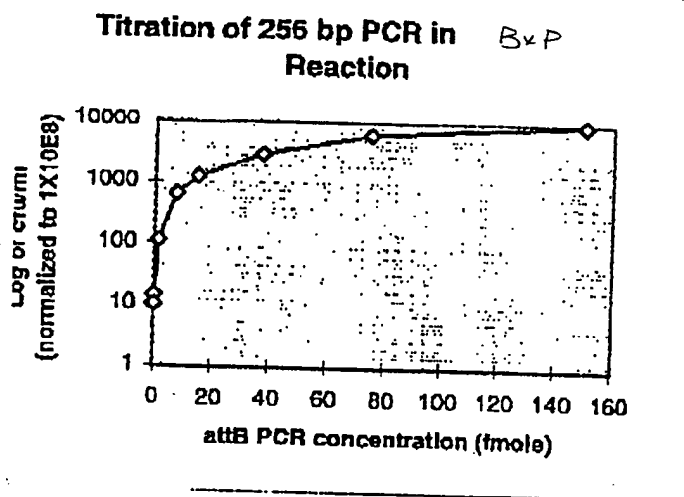


FIGURE 68

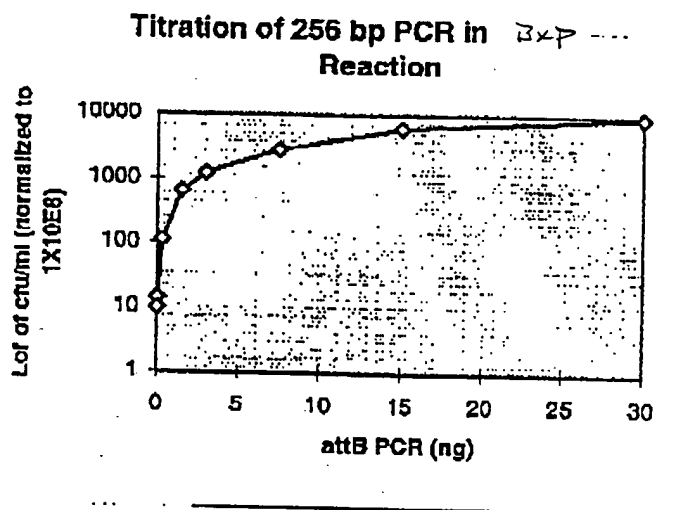
178/240

FIGURE
69

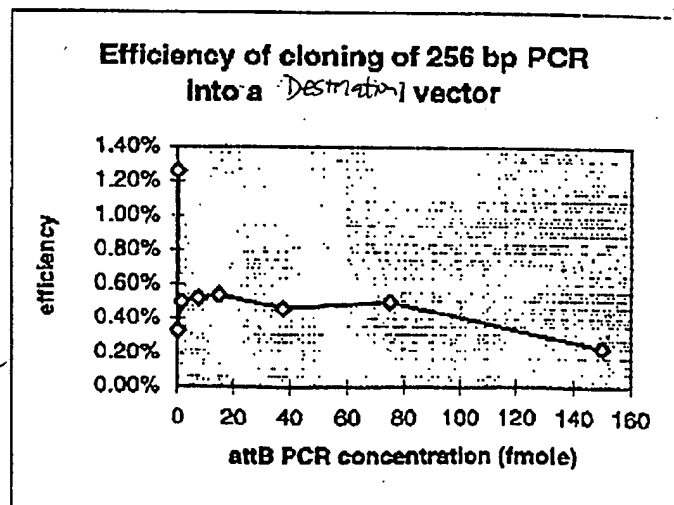
A



B



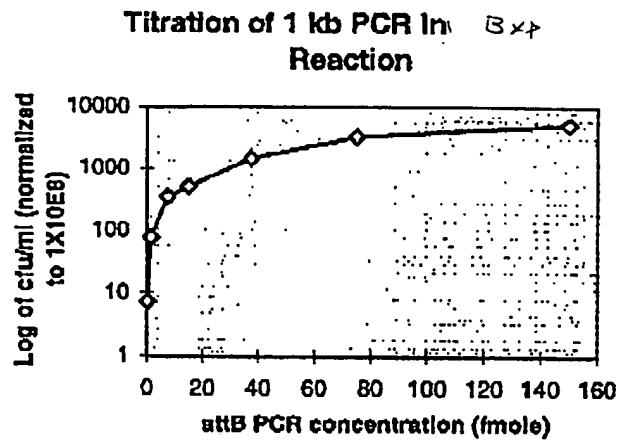
C



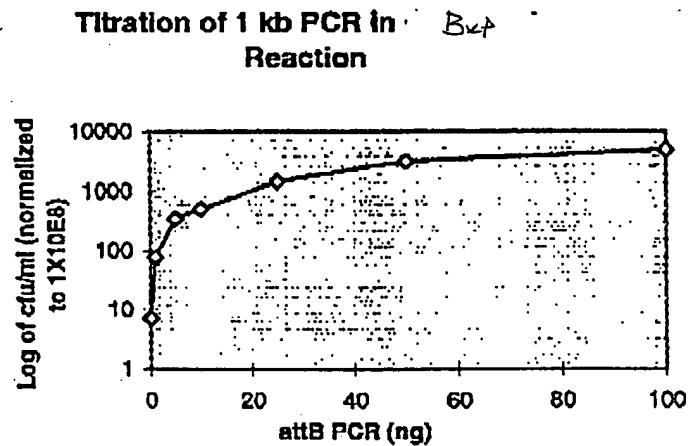
179/240

FIGURE
70

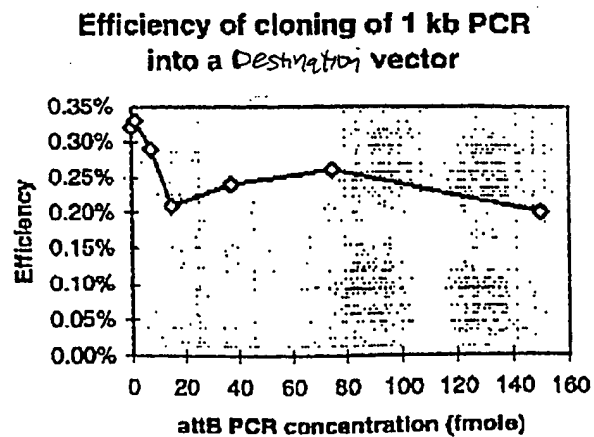
A



B



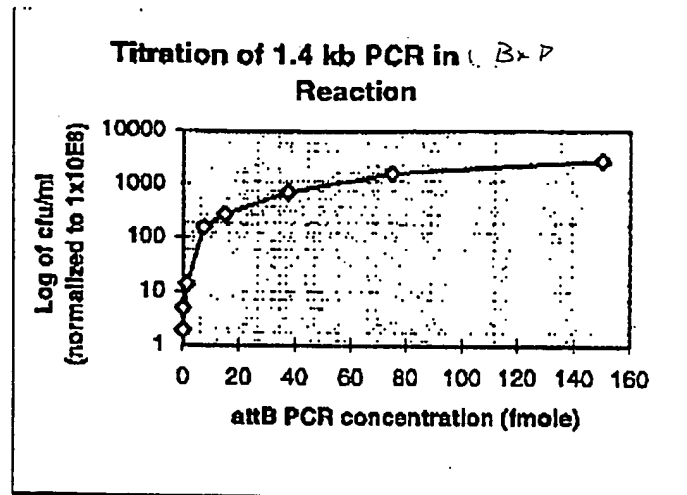
C



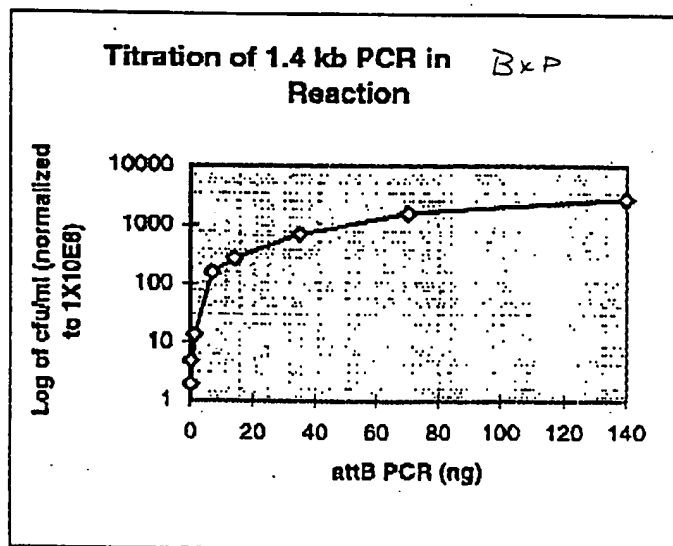
180/240

FIGURE 71

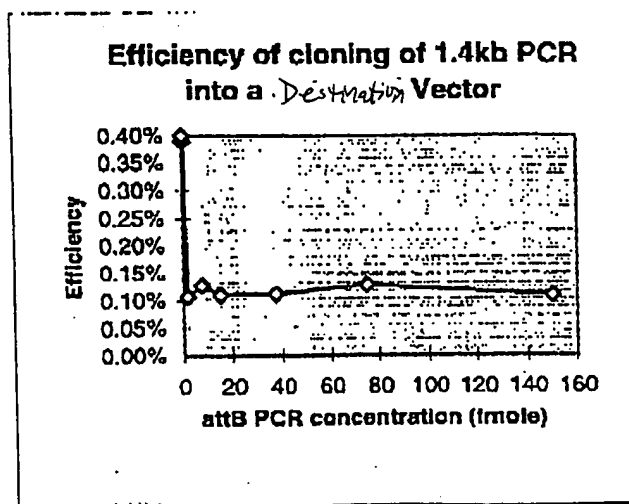
A



B



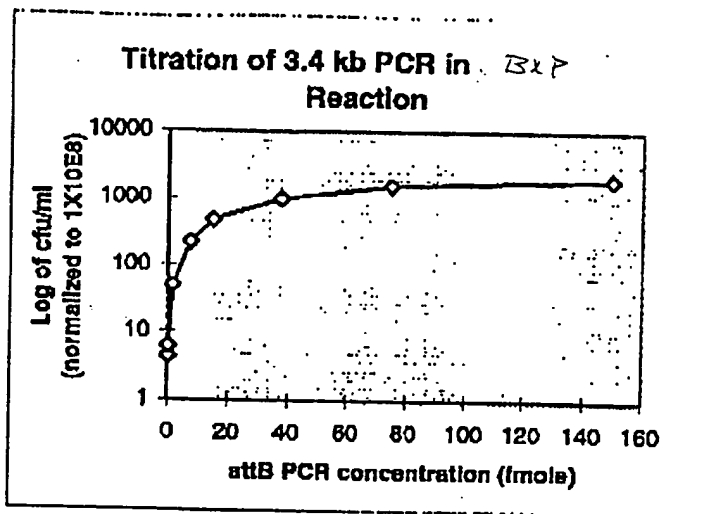
C



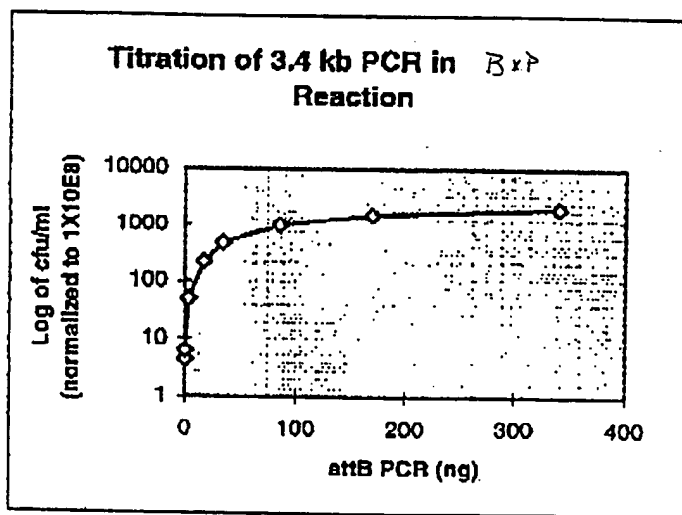
181/240

FIGURE 72

A



B



C

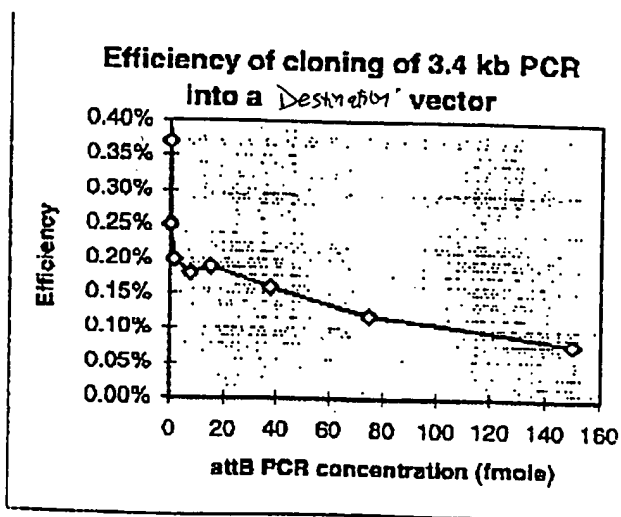
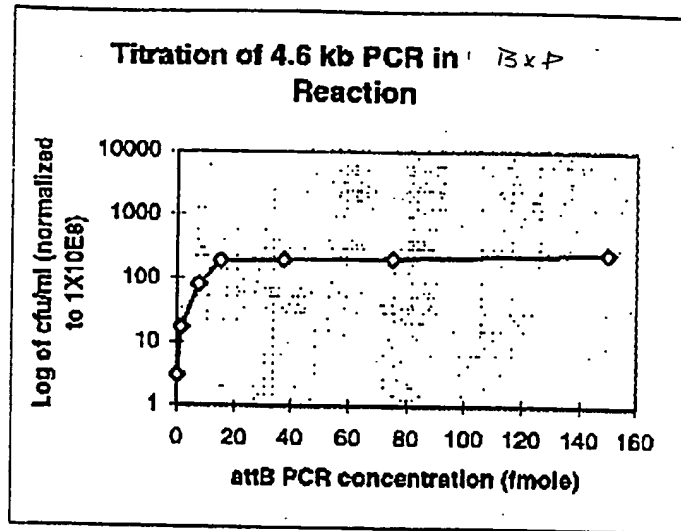
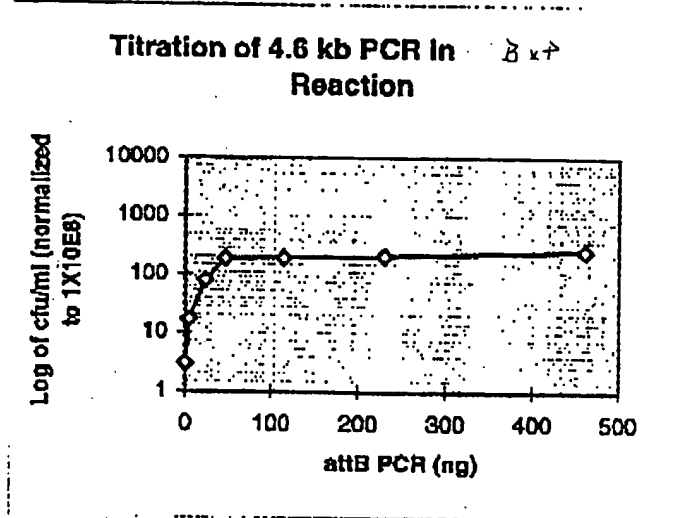


FIGURE 73

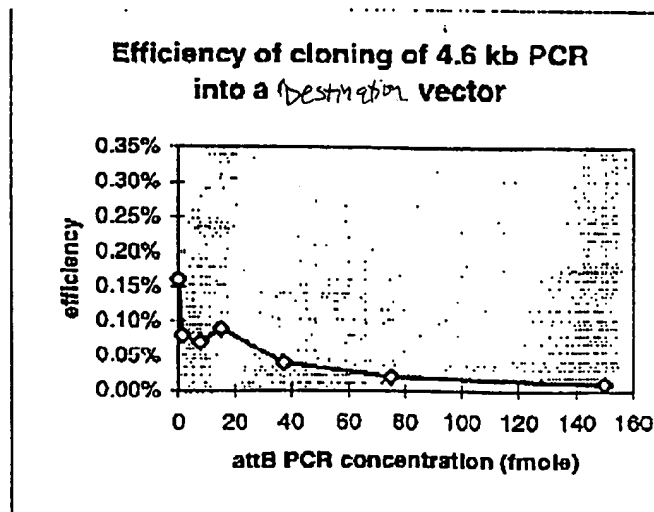
A



B



C



6.9 kb PCR DNA Titration in α BxP Reaction

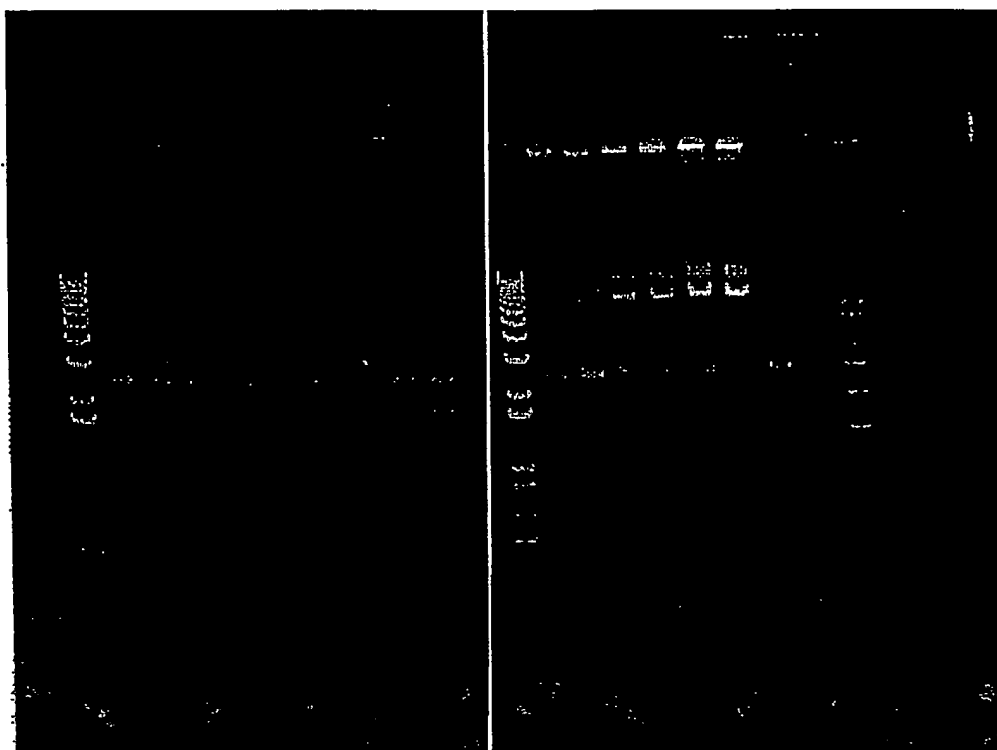


FIGURE 74

184/240

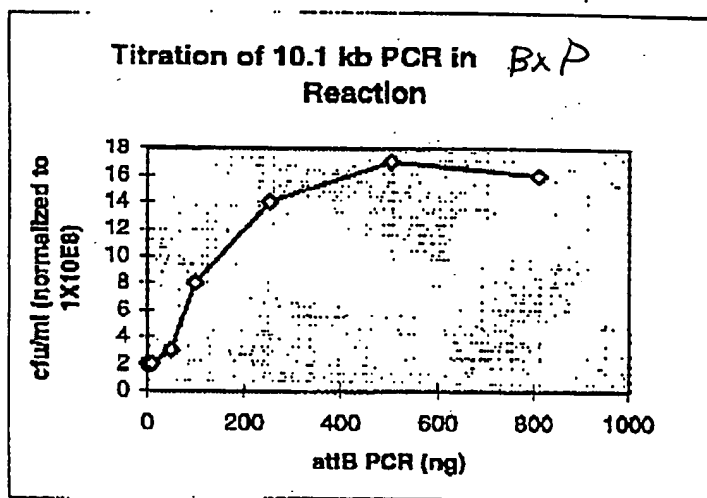


FIGURE 75-

185/240

10.1 kb PCR DNA Titration in Bx7 Reaction

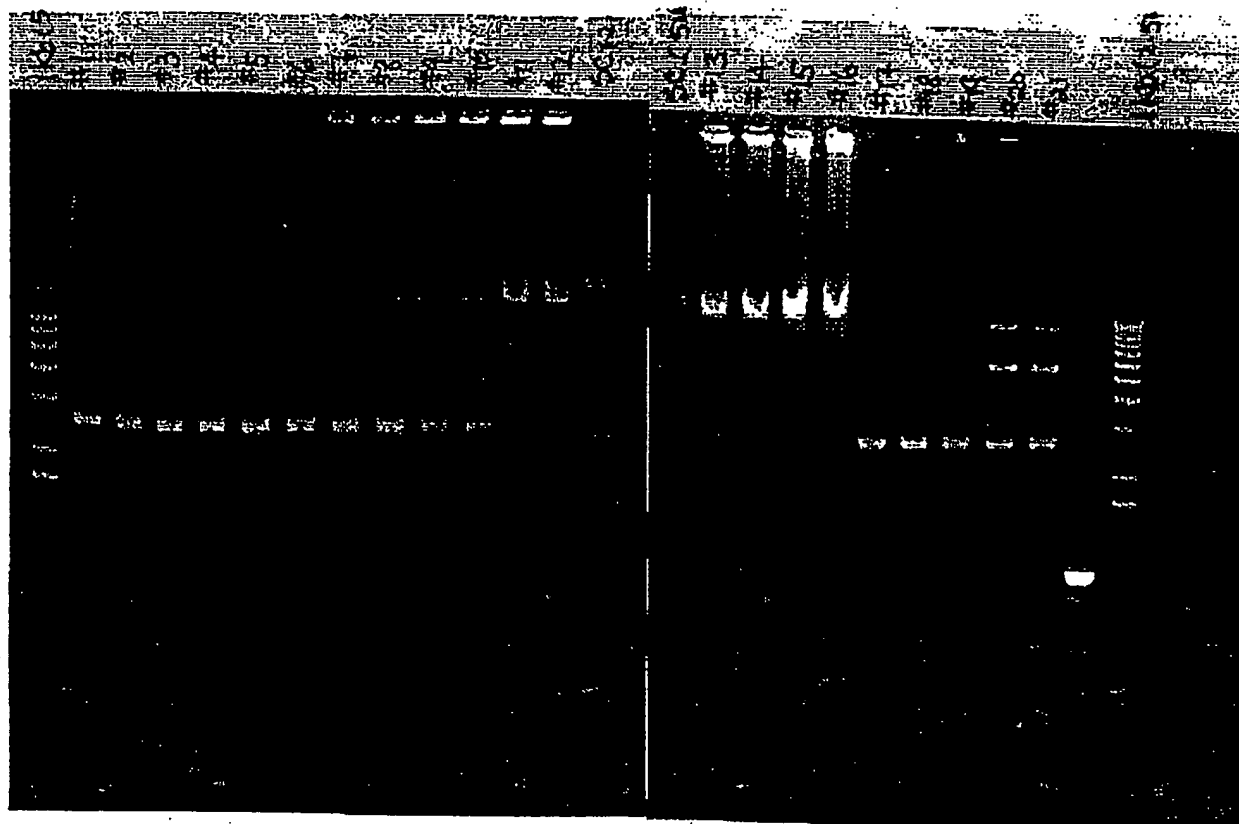


FIGURE 76

**Cloning of PCR Products of Different Sizes with the
GATEWAY™ PCR Cloning System**

Size	fmols PCR DNA	ng PCR DNA	Cols/ml Transformation (pUC=10 ⁸ CFU/ml)	Correct Clones/Total Examined**
0.26 kb*	15	3	1223	10/10 (a)
	37.5	7.5	2815	
1.0 kb	15	10	507	49/50 (b)
	37.5	25	1447	
1.4 kb	15	14	271	48/50 (c)
	37.5	35	683	
3.4 kb	15	34	478	9/10 (a)
	37.5	85	976	
4.6 kb	15	46	190	10/10 (a)
	37.5	115	195	
6.9 kb	15	69	30 (235)**	47/50 (b)
	37.5	173	54 (463)**	

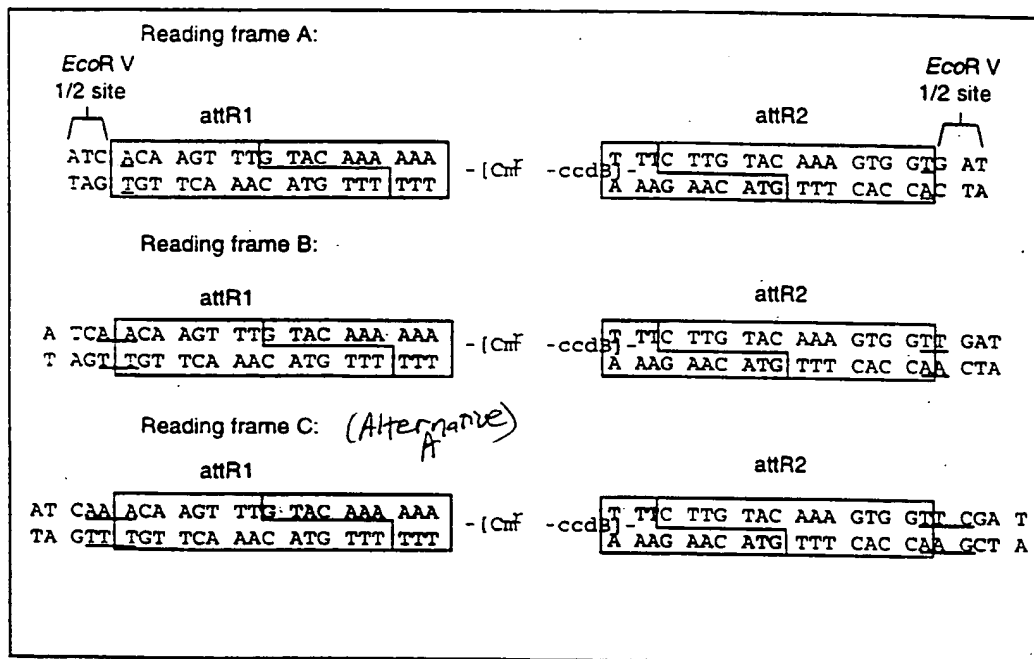
*The 0.26 kb PCR product was used unpurified; all the others were purified by precipitation with PEG/MgCl₂ as described in the text of Example 9, to remove primer dimers potentially present. Standard incubations were for 60 min.

**overnight incubation

- (a) DNA minipreps
- (b) ampR/kanR
- (c) tetR/kanR

Figure 77

187/240



Reading frame C: (Alternative)
B

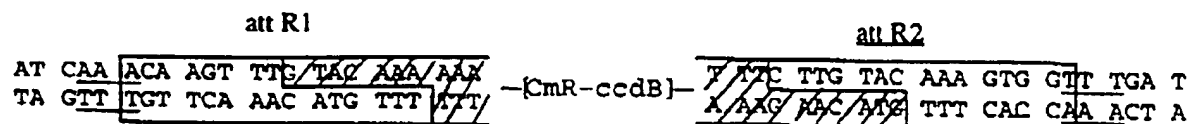


FIGURE 78

188/260

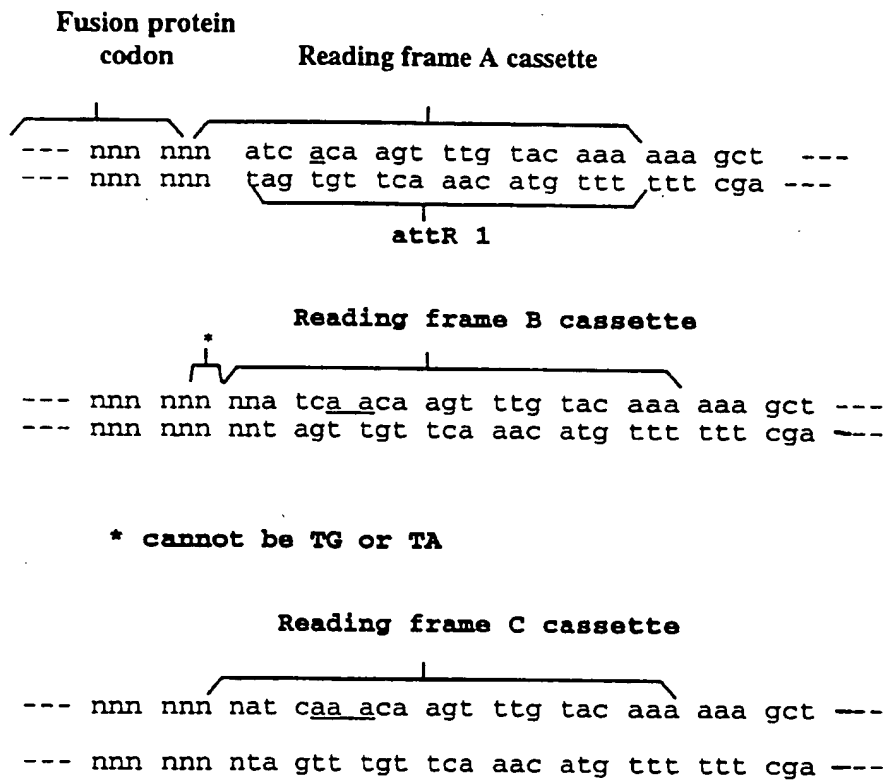


FIGURE 79

189/240

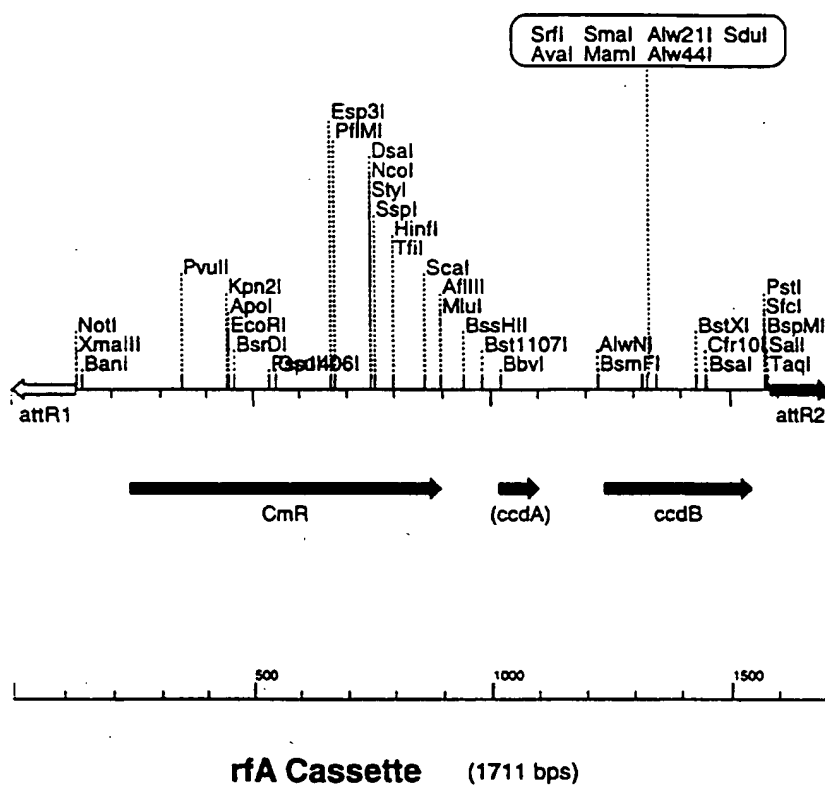


FIGURE 80

190/240

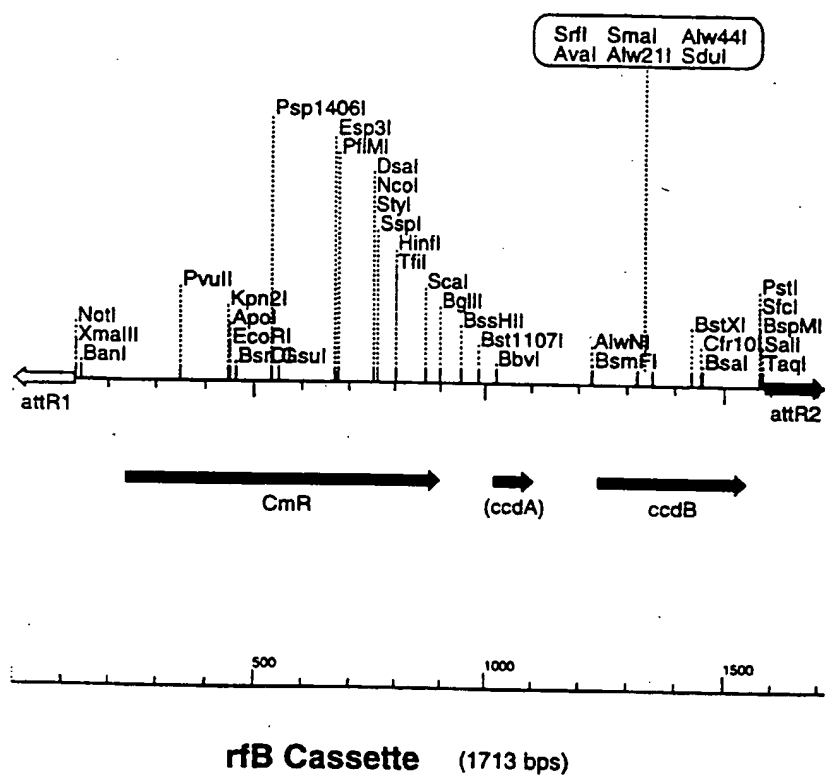
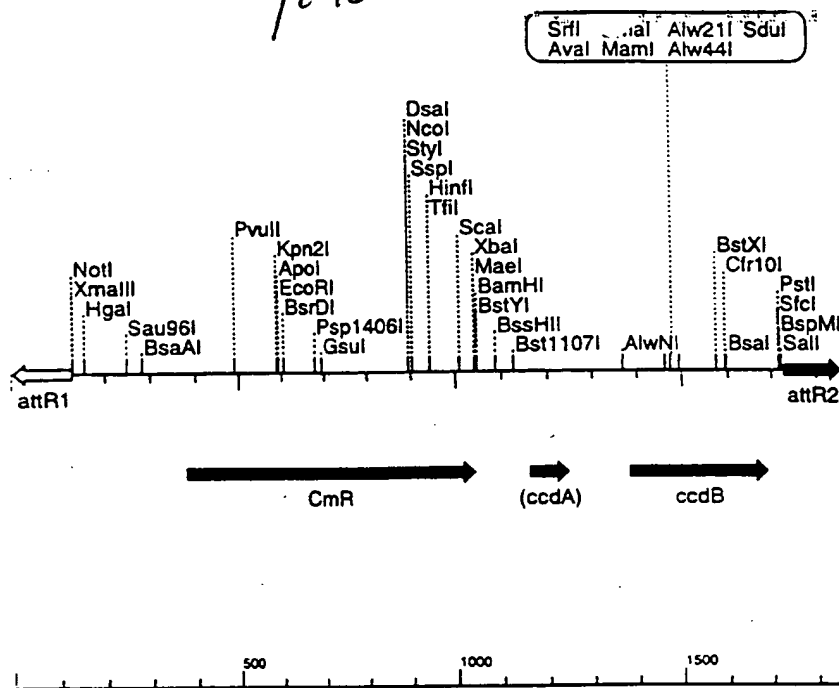


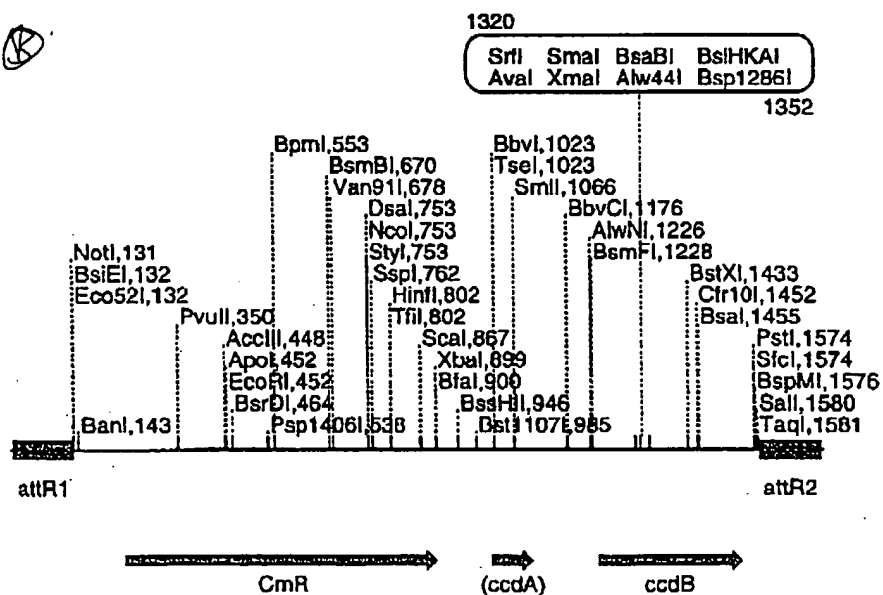
FIGURE 81

(A)



rFC Cassette (1856 bps)

(B)



rFC cassette (1715 bps)

FIGURE 82

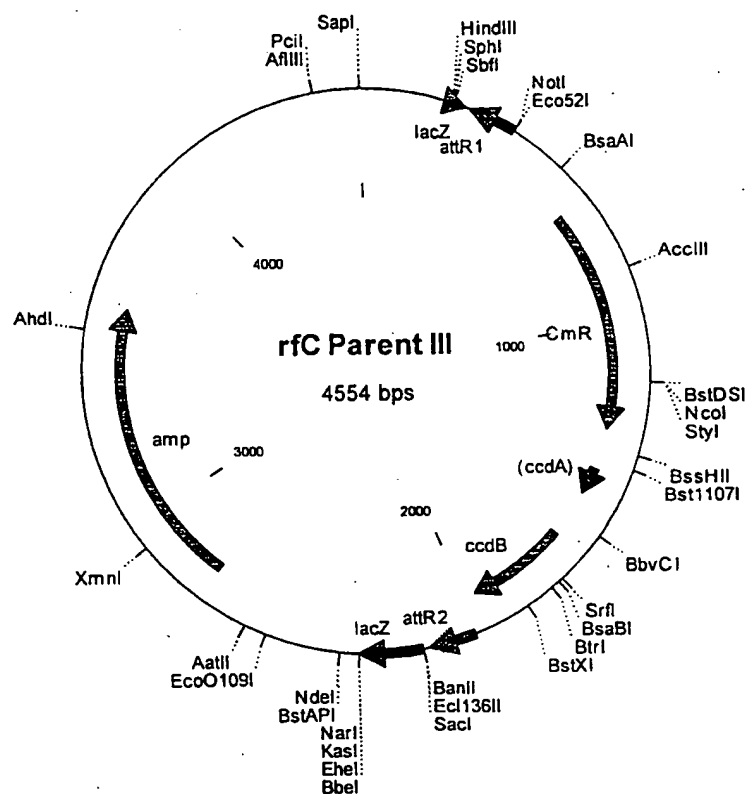


FIGURE 83A

prfC Parent III 4554 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
410..286		attR1
660..1319		CmR
1439..1523		inactivated ccdA
1661..1966		ccdB
2007..2131		attR2
2753..3613		amp

1	GCGCCCAATA	CGCAAACCGC	CTCTCCCCGC	GCGTTGGCCG	ATTCATTAAT	GCAGCTGGCA
61	CGACAGGTTT	CCCGACTGGA	AAGCGGGCAG	TGAGCGCAAC	GCAATTAATG	TGAGTTAGCT
121	CACTCATTAG	GCACCCAGG	CTTTACACTT	TATGCTTCCG	GCTCGTATGT	TGTGTGGAAT
181	TGTGAGCGGA	TAACAATTTT	ACACAGGAAA	CAGCTATGAC	CATGATTACG	CCAAGCTTGC
241	ATGCCTGCAG	GTCGACTCTA	GAGGATCCCC	GGGTACCGAT	ATCAAACAAG	TTTGTACAAA
301	AAAGCTGAAC	GAGAAACGTA	AAATGATATA	AATATCAATA	TATTAAATTA	GATTTTGCAT
361	AAAAAACAGA	CTACATAATA	CTGTAAAACA	CAACATATCC	AGTCACTATG	GCGGCCGCTA
421	AGTTGGCAGC	ATCACCCGAC	GCACCTTTGCG	CCGAATAAAT	ACCTGTGACG	GAAGATCACT
481	TCGCAGAATA	AATAAATCCT	GGTGTCCCTG	TTGATACCGG	GAAGCCCTGG	GCCAACTTTT
541	GGCGAAAATG	AGACGTTGAT	CGGCACGTAA	GAGGTTCCAA	CTTTCACCAT	AATGAAATAA
601	GATCACTACC	GGGCGTATTT	TTTGAGTTAT	CGAGATTTTC	AGGAGCTAAG	GAAGCTAAAA
661	TGGAGAAAAA	AATCACTGGA	TATACCACCG	TTGATATATC	CCAATGGCAT	CGTAAAGAAC
721	ATTTTGAGGC	ATTTTCAGTCA	GTTGCTCAAT	GTACCTATAA	CCAGACCGTT	CAGCTGGATA
781	TTACGGCCTT	TTTAAAGACC	GTAAGAAAAA	ATAAGCACAA	GTTTTATCCG	GCCTTTATTC
841	ACATTCTTGC	CCGCCTGATG	AATGCTCATC	CGGAATTCCG	TATGGCAATG	AAAGACGGTG
901	AGCTGGTGAT	ATGGGATAGT	GTTCAACCCTT	GTTACACCGT	TTTCCATGAG	CAAACCTGAAA
961	CGTTTTTCATC	GCTCTGGAGT	GAATACCACG	ACGATTTCCG	GCAGTTTCTA	CACATATATT
1021	CGCAAGATGT	GGCGTGTAC	GGTGAAAACC	TGGCCTATTT	CCCTAAAGGG	TTTATTGAGA
1081	ATATGTTTTT	CGTCTCAGCC	AATCCCTGGG	TGAGTTTCAC	CAGTTTTGAT	TTAAACGTGG
1141	CCAATATGGA	CAACTTCTTC	GCCCCCGTTT	TCACCATGGG	CAAATATTAT	ACGCAAGGCG
1201	ACAAGGTGCT	GATGCCGCTG	GCGATTACAG	TTCATCATGC	CGTCTGTGAT	GGCTTCCATG
1261	TCGGCAGAAT	GCTTAATGAA	TTACAACAGT	ACTGCGATGA	GTGGCAGGGC	GGGGCGTAAT
1321	CTAGAGGATC	CGGCTTACTA	AAAGCCAGAT	AACAGTATGC	GTATTTGCGC	GCTGATTTTT
1381	GCGGTATAAG	AATATATACT	GATATGTATA	CCCGAAGTAT	GTCAAAAAGA	GGTGTGCTAT
1441	GAAGCAGCGT	ATTACAGTGA	CAGTTGACAG	CGACAGCTAT	CAGTTGCTCA	AGGCATATAT
1501	GATGTCAATA	TCTCCGGTCT	GGTAAGCACA	ACCATGCAGA	ATGAAGCCCG	TCGCTCGCGT
1561	GCCGAACGCT	GGAAAGCGGA	AAATCAGGAA	GGGATGGCTG	AGGTCGCCCC	GTTTTATTGAA
1621	ATGAACGGCT	CTTTTGCTGA	CGAGAACAGG	GACTGGTGAA	ATGCAGTTTA	AGGTTTACAC
1681	CTATAAAAGA	GAGAGCCGTT	ATCGTCTGTT	TGTGGATGTA	CAGAGTGATA	TTATTGACAC
1741	GCCCCGGCGA	CGGATGGTGA	TCCCCCTGGC	CAGTGCACGT	CTGCTGTCAG	ATAAAGTCTC
1801	CCGTGAACCT	TACCCGGTGG	TGCATATCGG	GGATGAAAGC	TGGCGCATGA	TGACCACCGA
1861	TATGCCAGT	GTGCCGGTCT	CCGTTATCGG	GGAAGAAGTG	GCTGATCTCA	GCCACCGCGA
1921	AAATGACATC	AAAAACGCCA	TTAACCTGAT	GTTCTGGGGA	ATATAAATGT	CAGGCTCCGT
1981	TATACACAGC	CAGTCTGCAG	GTGACCATTA	GTGACTGGAT	ATGTTGTGTT	TTACAGTATT
2041	ATGTAGTCTG	TTTTTTATGC	AAAATCTAAT	TTAATATATT	GATATTTATA	TCATTTTACG
2101	TTTCTCGTTC	AGCTTTCTTG	TACAAAGTGG	TTCGATATCG	GTACCGAGCT	CGAATTCAC
2161	GGCCGTCGTT	TTACAACGTC	GTGACTGGGA	AAACCCTGGC	GTTACCCAAC	TTAATCGCCT
2221	TGCAGCACAT	CCCCCTTTCG	CCAGCTGGCG	TAATAGCGAA	GAGGCCCGCA	CCGATCGCCC
2281	TTCCCAACAG	TTGCGCAGCC	TGAATGGCGA	ATGGCGCCTG	ATGCGGTATT	TTCTCCTTAC
2341	GCATCTGTGC	GGTATTTTAC	ACCGCATATG	GTGCACTCTC	AGTACAATCT	GCTCTGATGC
2401	CGCATAGTTA	AGCCAGCCCC	GACACCCGCC	AACACCCGCT	GACGCGCCCT	GACGGGCTTG
2461	TCTGCTCCCG	GCATCCGCTT	ACAGACAAGC	TGTGACCGTC	TCCGGGAGCT	GCATGTGTCA
2521	GAGGTTTTCA	CCGTCACTAC	CGAAACGCCG	GAGACGAAAG	GGCCTCGTGA	TACGCCTATT
2581	TTTATAGGTT	AATGTCATGA	TAATAATGGT	TTCTTAGACG	TCAGGTGGCA	CTTTTCGGGG
2641	AAATGTGCGC	GGAACCCCTA	TTTGTATTAT	TTTCTAAATA	CATTCAAATA	TGTATCCGCT
2701	CATGAGACAA	TAACCTGAT	AAATGCTTCA	ATAATATTGA	AAAAGGAAGA	GTATGAGTAT
2761	TCAACATTTT	CGTGTGCCCC	TTATTCCTTT	TTTTGCGGCA	TTTTGCCTTC	CTGTTTTTGC

2821 TCACCCAGAA ACGCTGGTGA AAGTAAAAGA TGCTGAAGAT CAGTTGGGTG CACGAGTGGG
2881 TTACATCGAA CTGGATCTCA ACAGCGGTAA GATCCTTGAG AGTTTTCGCC CCGAAGAACG
2941 TTTTCCAATG ATGAGCACTT TTAAAGTTCT GCTATGTGGC GCGGTATTAT CCCGTATTGA
3001 CGCCGGGCAA GAGCAACTCG GTCGCCGCAT ACACTATTCT CAGAATGACT TGGTTGAGTA
3061 CTCACCAGTC ACAGAAAAGC ATCTTACGGA TGGCATGACA GTAAGAGAAT TATGCAGTGC
3121 TGCCATAACC ATGAGTGATA ACACTGCGGC CAACTTACTT CTGACAACGA TCGGAGGACC
3181 GAAGGAGCTA ACCGCTTTTT TGCACAACAT GGGGGATCAT GTAAC TCGCC TTGATCGTTG
3241 GGAACCGGAG CTGAATGAAG CCATACCAA CGACGAGCGT GACACCACGA TGCTGTAGC
3301 AATGGCAACA ACGTTGCGCA AACTATTAAC TGGCGAACTA CTTACTCTAG CTTCCTGGCA
3361 ACAATTAATA GACTGGATGG AGGCGGATAA AGTTGCAGGA CCACTTCTGC GCTCGGCCCT
3421 TCCGGCTGGC TGGTTTATTG CTGATAAATC TGGAGCCGGT GAGCGTGGGT CTCGCGGTAT
3481 CATTGCAGCA CTGGGGCCAG ATGGTAAGCC CTCCCGTATC GTAGTTATCT ACACGACGGG
3541 GAGTCAGGCA ACTATGGATG AACGAAATAG ACAGATCGCT GAGATAGGTG CCTCACTGAT
3601 TAAGCATTGG TAACTGTCAG ACCAAGTTTA CTCATATATA CTTTAGATTG ATTTAAAACT
3661 TCATTTTAA TTTAAAAGGA TCTAGGTGAA GATCCTTTT GATAATCTCA TGACCAAAT
3721 CCCTTAACGT GAGTTTTCGT TCCACTGAGC GTCAGACCCC GTAGAAAAGA TCAAAGGATC
3781 TTCTTGAGAT CCTTTTTTTC TGCGCGTAAT CTGCTGCTTG CAAACAAAAA AACCACCGCT
3841 ACCAGCGGTG GTTTGTTTGC CGGATCAAGA GCTACCACT CTTTTTCCGA AGGTAACCTG
3901 CTTCAGCAGA GCGCAGATAC CAAATACTGT CTTCTAGTG TAGCCGTAGT TAGGCCACCA
3961 CTTCAAGAAC TCTGTAGCAC CGCCTACATA CCTCGCTCTG CTAATCCTGT TACCAGTGGC
4021 TGCTGCCAGT GGCAGATAAGT CGTGTCTTAC CGGGTTGGAC TCAAGACGAT AGTTACCGGA
4081 TAAGGCGCAG CGGTCGGGCT GAACGGGGGG TTCGTGCACA CAGCCCAGCT TGGAGCGAAC
4141 GACCTACACC GAACTGAGAT ACCTACAGCG TGAGCTATGA GAAAGCGCCA CGCTTCCCGA
4201 AGGGAGAAAAG GCGGACAGGT ATCCGGTAAG CGGCAGGGTC GGAACAGGAG AGCGCACGAG
4261 GGAGCTTCCA GGGGGAAACG CCTGGTATCT TTATAGTCCT GTCGGGTTTC GCCACCTCTG
4321 ACTTGAGCGT CGATTTTGT GATGCTCGTC AGGGGGGCGG AGCCTATGGA AAAACGCCAG
4381 CAACGCGGCC TTTTACGGT TCCTGGCCTT TTGCTGGCCT TTTGCTCACA TGTTCTTTCC
4441 TCGCTTATCC CCTGATTCTG TGGATAACCG TATTACCGCC TTTGAGTGAG CTGATACCGC
4501 TCGCCGACG CGAACGACCG AGCGCAGCGA GTCAGTGAGC GAGGAAGCGG AAGA

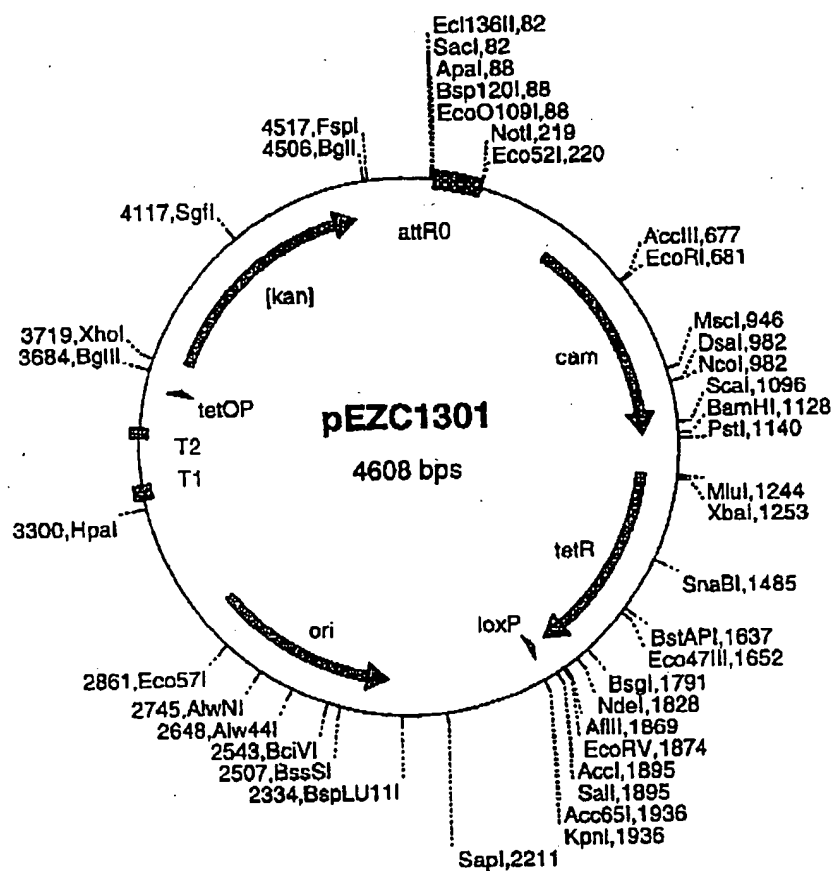


FIGURE 84

196/240

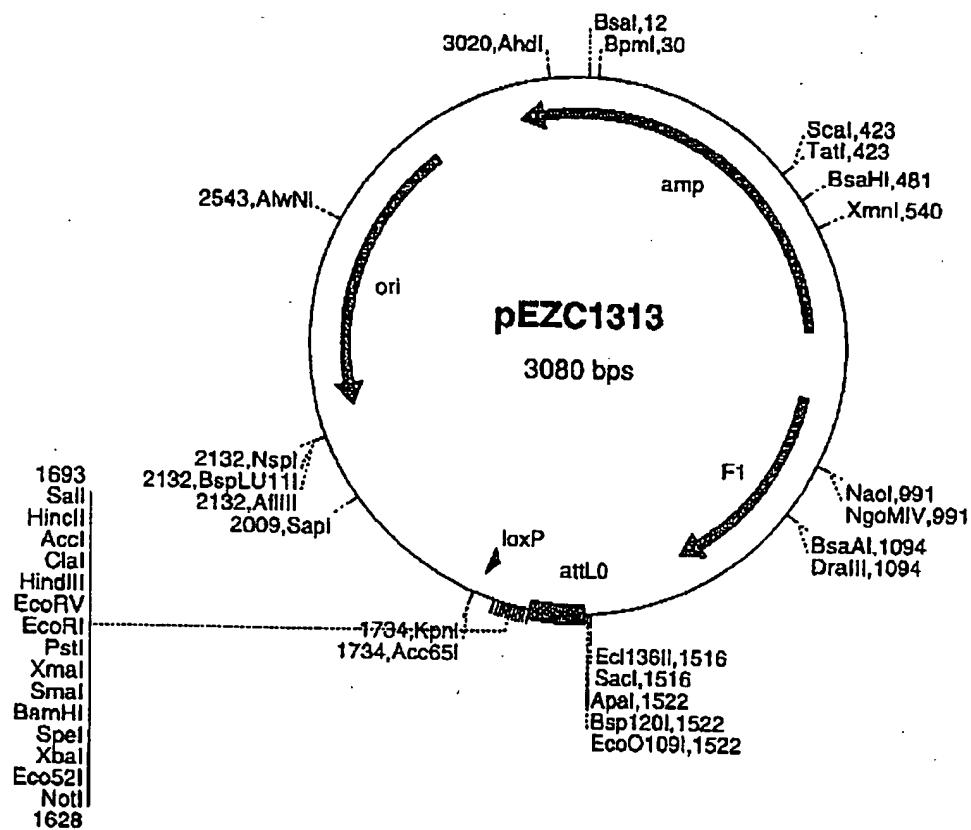


FIGURE 85

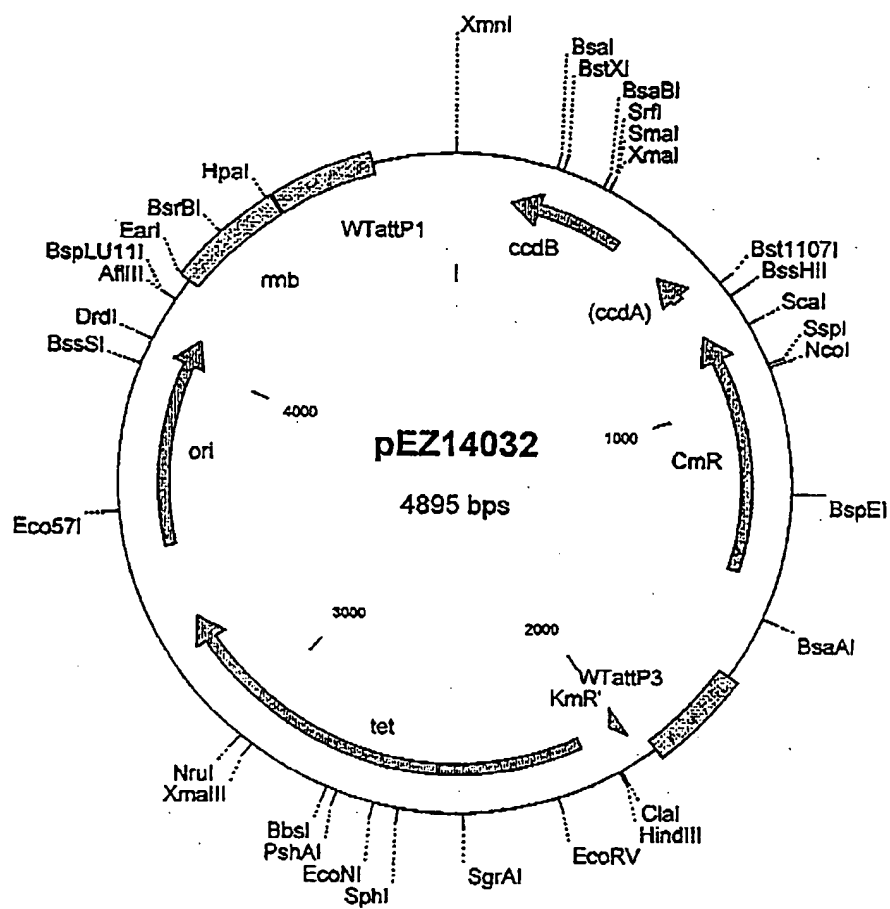


FIGURE 86

198/260

FIGURE 87

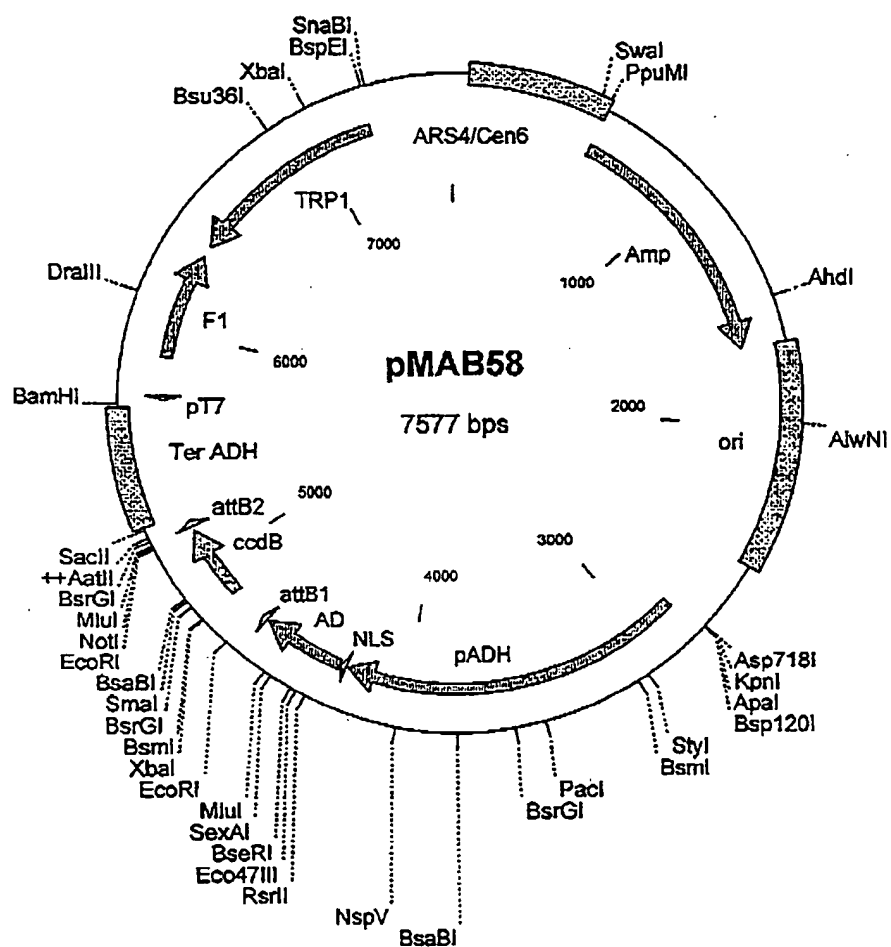
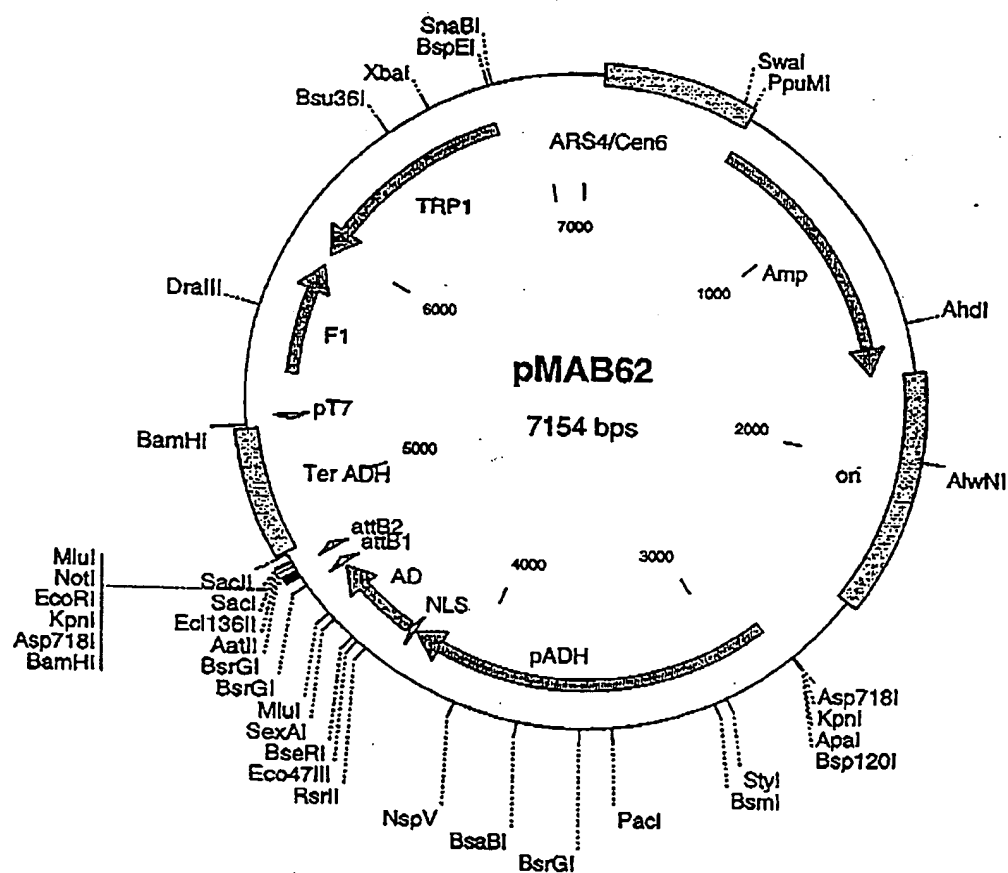
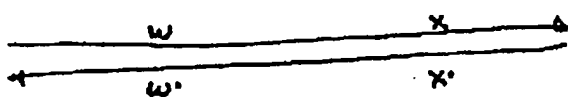


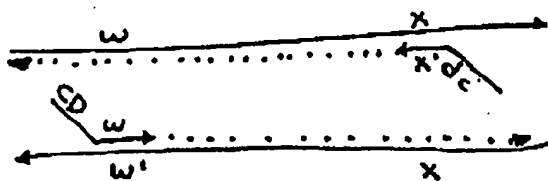
FIGURE 88



DNA to be amplified (5' → 3'):



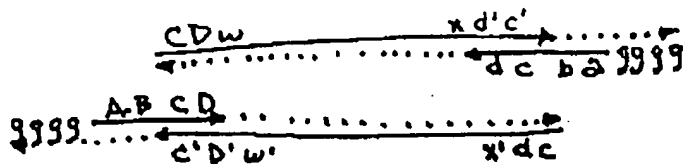
↓ Denature, anneal
hybrid primers,
↓ extend with polymerase



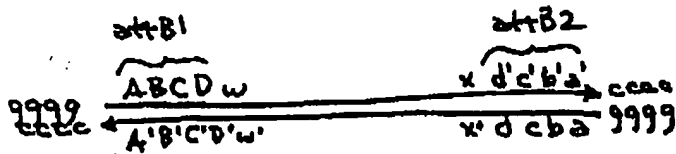
↓ amplification cycles



↓ Denature, anneal
attB primers,
extend with polymerase



↓ amplification cycles



attB1 primer:

9999 \xrightarrow{ABCD}

attB2 primer:

9999 $\xrightarrow{abc d}$

Hybrid primers (part
attB, part gene
specific):

\xrightarrow{CDw}

$\xrightarrow{cd x'}$

FIGURE 89

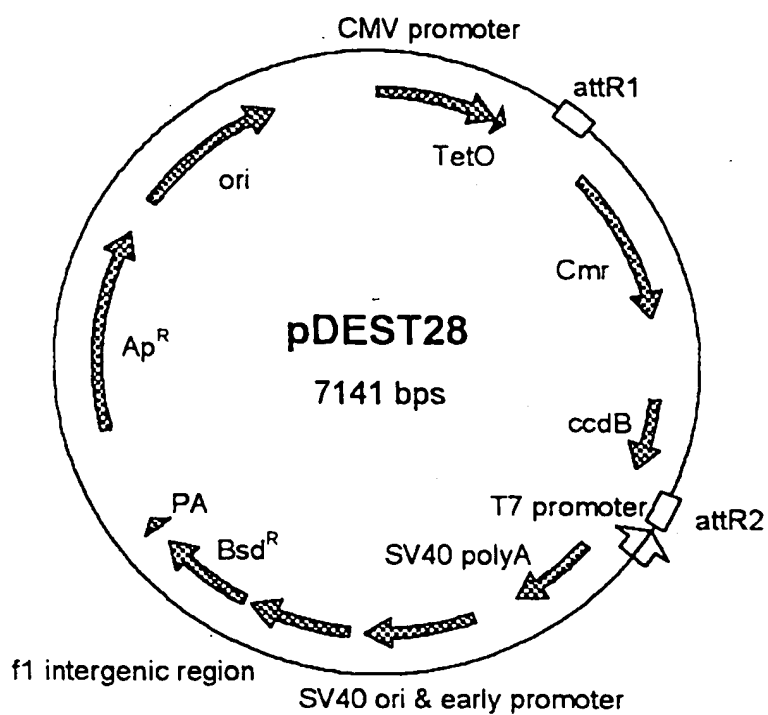


FIGURE 90A

pDEST28 7141 bp

ATGCATGTCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCC
CGCCCATTTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCAT
TGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTAT
CATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTAT
GCCCAGTACATGACCTTATGGGACTTTCTACTTGGCAGTACATCTACGTATTAGTCATC
GCTATTACCATGGTGTATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTTGAC
TCACGGGGATTTCGAAGTCTCCACCCCAATTGACGTCAATGGGAGTTTGTGGTGGCACC
AATCAACGGGACTTTCCAAAATGTCGTAACAACCTCCGCCCCATTGACGCAAATGGGCGGT
AGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCTCCCTATCAGTGATAGAGATCTC
CCTATCAGTGATAGAGATCGTTCGACGAGCTCGTTTGTAGTGAACCGTCAGATCGCCTGGAGA
CGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCCAGCCTCCGGACT
CTAGAGGATCCCTACCGGTGATATCCTCGAGCCCATCAACAAGTTTGTACAAAAAAGCTG
AACGAGAAACGTAAAATGATATAAATATCAATATATTAAATTAGATTTTGCATAAAAAAC
AGACTACATAATACTGTAAACACAACATATCCAGTCACTATGGCGGCCCGCATTAGGCAC
CCCAGGCTTTACACTTTATGCTTCCGGCTCGTATAATGTGTGGATTTTGTAGTTAGGATCC
GGCGAGATTTTCAGGAGCTAAGGAAGCTAAATGGAGAAAAAATCACTGGATATACCAC
CGTTGATATATCCCAATGGCATCGTAAAGAACATTTTGAGGCATTTTCAGTCAGTTGCTCA
ATGTACCTATAACCAGACCGTTTCAGCTGGATATTACGGCCTTTTTAAAGACCGTAAAGAA
AAATAAGCACAAAGTTTTATCCGGCCTTTATTCACATTCTTGCCCGCCTGATGAATGCTCA
TCCGGAATTCCGTATGGCAATGAAAGACGGTGAGCTGGTGATATGGGATAGTGTTACCCC
TTGTTACACCGTTTTCCATGAGCAAACCTGAAACGTTTTTCATCGCTCTGGAGTGAATACCA
CGACGATTTCCGGCAGTTTTCTACACATATATTGCAAGATGTGGCGTGTTACGGTGAAAA
CCTGGCCTATTTCCTTAAAGGGTTTTATTGAGAATATGTTTTCTGCTCTCAGCCAATCCCTG
GGTGAGTTTTCACCAAGTTTTGATTTAAACGTGGCCAATATGGACAACCTTCTTCGCCCCCGT
TTTCACCATGGGCAAATATTATACGCAAGGCGACAAGGTGCTGATGCCGCTGGCGATTCA
GGTTCATCATGCCGTCTGTGATGGCTTCCATGTCCGCAGAATGCTTAATGAATTACAACA
GTACTGCGATGAGTGGCAGGGCGGGGCGTAAAGATCTGGATCCGGCTTACTAAAAGCCAG
ATAACAGTATGCGTATTTGCGCGCTGATTTTTGCGGTATAAGAATATATACTGATATGTA
TACCCGAAGTATGTCAAAAAGAGGTGTGCTATGAAGCAGCGTATTACAGTGACAGTTGAC
AGCCACAGCTATCAGTTGCTCAAGGCATATGATGTCAATATCTCCGGTCTGGTAAGCA
ACACCATGCAGAAATGAAGCCCGTCTGCTGCGTCCGAACGCTGGAAAGCGGAAAAATCAGG
AAGGGATGGCTGAGGTGCGCCCGTTTTATTGAAATGAACGGCTCTTTTGCTGACGAGAACA
GGGACTGGTGAAATGCAGTTTAAGGTTTACACCTATAAAAGAGAGAGCCGTTATCGTCTG
TTTGTGGATGTACAGAGTGATATTATTGACACGCCCCGGCGACGGATGGTGATCCCCCTG
GCCAGTGACAGTCTGCTGTGATGATAAAGTCTCCCGTGAACCTTTACCCGGTGGTGATATC
GGGGATGAAAGCTGGCGCATGATGACCACCGATATGGCCAGTGTGCCGGTCTCCGTTATC
GGGGAAGAAGTGGCTGATCTCAGCCACCGCGGAAAATGACATCAAAAACGCCATTAACCTG
ATGTTCTGGGGAATATAAATGTCAGGCTCCCTTATACACAGCCAGTCTGCAGGTCGACCA
TAGTGACTGGATATGTTGTGTTTTTACAGTATTATGATGTAGTCTGTTTTTTTATGCAAAATCTA
ATTTAATATATTGATATTTATATCATTTTTACGTTTCTCGTTTCAGCTTTCTTGACAAAGT
GGTTGATGGGCGGCCGCTCTAGAGGGCCCAAGCTTACGCGTGCATGCGACGTCATAGCTC
TCTCCCTATAGTGAGTCGTATTATAAGCTAGGCACTGGCCGTGTTTTTACAACGTCGTGA
CTGGGAAAACCTGCTAGCTTGGGATCTTTGTGAAGGAACCTTACTTCTGTGGTGTGACATA
ATTGGACAAACTACCTACAGAGATTTAAAGCTCTAAGGTAAATATAAAATTTTTAAGTGT
ATAATGTGTTAAACTAGCTGCATATGCTTGTGCTTGAGAGTTTTGCTTACTGAGTATGA
TTTATGAAAATATTATACACAGGAGCTAGTGATTCTAATTGTTTGTGTATTTAGATTCA
CAGTCCCAAGGCTCATTTTCAGGCCCCCTCAGTCCTCACAGTCTGTTTCATGATCATAATCAG
CCATACCACATTTGTAGAGGTTTTACTTGCTTTAAAAAACCTCCACACCTCCCCCTGAA
CCTGAAACATAAAATGAATGCAATTGTTGTTGTTAACTTGTTTTATTGCAGCTTATAATGG
TTACAAATAAAGCAATAGCATCACAAATTTACAAATAAAGCATTTTTTCACTGCATTCT
TAGTTGTGGTTTTGTCCAAACTCATCAATGTATCTTATCATGTCTGGATCGATCCTGCATT
AATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGGCGTATTGGCTGGCGTAATAGCGAAG
AGGCCGACCGGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGGACGCGC
CCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGTTTACGCGCAGCGTGACCGCTACAC
TTGCCAGCGCCCTAGCGCCCGCTCCTTTGCTTTCTTCCCTTCTTCTCGCCACGTTCCG
CCGCTTTTCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTT-

Figure 90B

TACGGCACCTCGACCCCCAAAAAAGCTTGATTAGGGTGATGGTTACGCTAGTGGGCCATCGC
CCTGATAGACGGTTTTTCGCCCTTTGACCGTTGGAGTCCACGTTCTTTAATAGTGGACTCT
TGTTCCAAACTGGAACAACACTCAACCTATCTCGGTCTATTCTTTTGATTTATAAGGGA
TTTTGCCGATTTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAATATTTAACGCGA
ATTTTAACAATAATTAACGTTTACAATTTTCGCTGATGCGGTATTTTCTCCTTACGCAT
CTGTGCGGTATTTACACCCGCATACGCGGATCTGCGCAGCACCATGGCCTGAAATAACCT
CTGAAAGAGGAAGCTTGGTTAGGTACCTTCTGAGGCGGAAAGAACCAGCTGTGGAATGTGT
GTCAGTTAGGGTGTGGAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGC
ATCTCAATTAGTCAGCAACCAGGTGTGGAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTA
TGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCCGCCCTAACTCCGCCCATCC
CGCCCCTAACTCCGCCAGTTCCGCCCATTTCCGCCCATGGCTGACTAATTTTTTTTA
TTTATGCAGAGGCCGAGGCCGCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCT
TTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTTGATTCTTCTGACACAACAGTCTCGAACT
TAAGACCATGGCCAAGCCTTTGTCTCAAGAAGAATCCACCCTCATTGAAAGAGCAACGGC
TACAATCAACAGCATCCCCATCTCTGAAGACTACAGCGTCGCCAGCGCAGCTCTCTCTAG
CGACGGCCGCATCTTCACTGGTGTCAATGTATATCATTTTACTGGGGGACCTTGTGCAGA
ACTCGTGGTGTGGGCACTGCTGCTGCTGCGGCAGCTGGCAACCTGACTTGTATCGTCGC
GATCGGAAATGAGAACAGGGGCATCTTGAGCCCCCTGCGGACGGTGCCGACAGGTCTTCT
CGATCTGCATCCTGGGATCAAAGCCATAGTGAAGGACAGTGATGGACAGCCGACGGCAGT
TGGGATTCTGTAATTGCTGCCCTCTGGTTATGTGTGGGAGGGCTAAGCACTTCGTGGCCG
AGTTCGAAATGACCGACCAAGCGACGCCCAACCTGCCATCACGATGGCCGCAATAAAATA
TCTTTATTTTCATTACATCTGTGTGTGGTTTTTTGTGTGAATCGATAGCGATAAGGATC
CGCGTATGGTGCATCTCTAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGCCCCGA
CACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTAC
AGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTGAGAGGTTTTACCGTCAACCG
AAACGCGCGAGACGAAAGGGCCTCGTGATACGCCCTATTTTTATAGGTTAATGTATGATA
ATAATGGTTTTCTTAGACGTGAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATT
TGTTTTATTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAA
ATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTGCGCCTT
ATTCCTTTTTTTCGGGCATTTTGCCTTCCTGTTTTTGTCTCACCAGAAACGCTGGTGAAA
GTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAAC
AGCGGTAAAGATCCTTTGAGAGTTTTTCGCCCCGAAGAAGCTTTTCCAATGATGAGCACTTTT
AAAGTTCGTCTATTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGT
CGCCGCATACACTATTCTCAGAAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCAT
CTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAAC
ACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCCTTTTTTG
CACAACATGGGGGATCATGTAACCTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCC
ATACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAA
CTATTAAGTGGGCAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAG
GCGGATAAAGTTGACGAGCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCT
GATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGAT
GGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAA
CGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGAC
CAAGTTTACTCATATATACTTTAGATTGATTTAAAGCTTCAATTTTAAATTTAAAGGATC
TAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTTCGTTT
CACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTG
CGCGTAATCTGCTGCTTGCAACAAAAAACCACCGCTACCAGCGGTGGTTTGTGTGCCG
GATCAAGAGTACCAACTCTTTTTCCGAAGGTAAGTGGCTTCAGCAGAGCGCAGATACCA
AATACTGTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCG
CCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCTG
TGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTGGGCTGA
ACGGGGGGTTTCGTGCACACAGCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATAC
CTACAGCGTGAGCATTGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTAT
CCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCC
TGGTATCTTTATAGTCTGTGCGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGA
TGCTCGTCAGGGGGCGGAGCCTATGGAAGAACGCCAGCAACGCGGCCTTTTACGGTTT
CTGGCCTTTTGTGCGCCTTTTGTCTACATGTTCTTCTGCGTTATCCCTGATTCTGTG
GATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGACGCCGAACGACCGAG-

FIGURE 90C

CGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCC
GCGCGTTGGCCGATTCATTAATGCAGAGCTTGCAATTCGCGCGTTTTTCAATATTATTGA
AGCATTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAAT
AAACAAATAGGGGTTCCGCGCACATTTCCCGAAAAGTGCCACCTGACGTCTAAGAAACC
ATTATTATCATGACATTACCTATAAAAATAGGCGTAGTACGAGGCCCTTCACTCATTA
G

FIGURE 90D

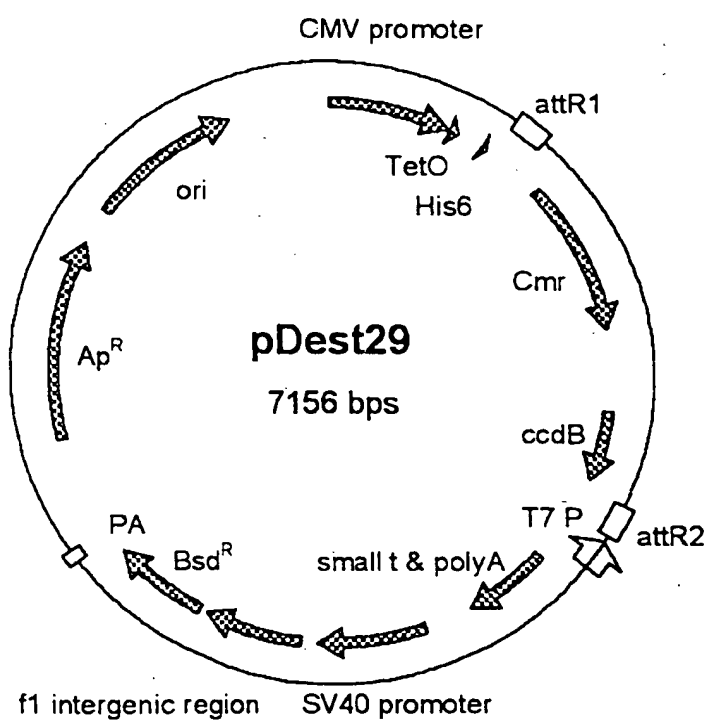


FIGURE 91 A

pDEST29 7156 bp

ATGCATGTCGTTACATAACTTACGGTAAATGGCCCCGCTGGCTGACCGCCCAACGACCCC
CGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCAT
TGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTAT
CATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCCGCTGGCATTAT
GCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATC
GCTATTACCATGGTGATGCGGTTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTTGAC
TCACGGGGATTTTCCAAGTCTCCACCCCATTTGACGTCAATGGGAGTTTTGTTTTGGCACCAA
AATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGT
AGGCGTGACGGTGGGAGGTCTATATAAGCAGAGCTCTCCCTATCAGTGATAGAGATCTC
CCTATCAGTGATAGAGATCGTTCGACGAGCTCGTTTTAGTGAACCGTCAGATCGCCTGGAGA
CGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCCAGCCTCCGGACC
ATGGCGTACTACCATCACCATCACCATCACACCGGTGATATCCTCGAGCCCATCACAAGT
TTGTACAAAAAAGCTGAACGAGAAACGTAATAATGATATAAATATCAATATATTAAATTAG
ATTTTGCATAAAAAACAGACTACATAATACTGTAAAACACAACATATCCAGTCACTATGG
CGGCCGCATTAGGCACCCCAAGGCTTTACACTTTATGCTTCCGGCTCGTATAATGTGTGGA
TTTTGAGTTAGGATCCGGCGAGATTTTCAGGAGCTAAGGAAGCTAAAATGGAGAAAAAAA
TCACTGGATATACCACCGTTGATATATCCCAATGGCATCGTAAAGAACATTTTGAGGCAT
TTCAGTCAGTTGCTCAATGTACCTATAACCAGACCGTTTCAGCTGGATATTACGGCCTTTT
TAAAGACCGTAAAGAAAAATAAGCACAAAGTTTTATCCGGCCTTTATTCACATTCTTGCCC
GCCTGATGAATGCTCATCCGGAATTCGGTATGGCAATGAAAGACGGTGAGCTGGTGATAT
GGGATAGTGTTTACCCTTGTACACCGTTTTTCCATGAGCAAACGTGAAACGTTTTTCATCGC
TCTGGAGTGAATACCACGACGATTTCCGGCAGTTTTCTACACATATATTCGCAAGATGTGG
CGTGTTACGGTGAAAACCTGGCCTATTTCCCTAAAGGGTTTTATTGAGAATATGTTTTTCG
TCTCAGCCAATCCCTGGGTGAGTTTACCAGTTTTGATTTAAACGTGGCCAATATGGACA
ACTTCTTCGCCCCCGTTTTTACCATGGGCAAATATTATACGCAAGGCGACAAGGTGCTGA
TGCCGCTGGCGATTTCAGGTTTCATCATGCCGTCTGTGATGGCTTCCATGTCCGCAGAATGC
TTAATGAATTACAACAGTACTGCGATGAGTGGCAGGGCGGGGCGTAAACGCGTGGATCCG
GCTTACTAAAAGCCAGATAACAGTATGCGTATTTGCGCGCTGATTTTTGCGGTATAAGAA
TATATACTGATATGTAACCCGAAGTATGTCAAAAGAGGTGTGCTATGAAGCAGCGTAT
TACAGTGACAGTTGACAGCGACAGCTATCAGTTGCTCAAGGCATATATGATGTCAATATC
TCCGGTCTGGTAAGCACAAACCATGCAGAATGAAGCCCGTCGTCTGCGTGCCGAACGCTGG
AAAGCGGAAAATCAGGAAGGGATGGCTGAGGTGCCCCGTTTATTGAAATGAACGGCTCT
TTTGCTGACGAGAACAGGGACTGGTGAAATGCAGTTTAAAGTTTACACCTATAAAAGAGA
GAGCCGTTATCGTCTGTTTGTGGATGTACAGAGTGATATTATTGACACGCCCCGGGCGACG
GATGGTGATCCCCCTGGCCAGTGACAGTCTGCTGTGATGATAAAGTCTCCCGTGAACTTTA
CCCCGTGGTGATATGTCGGGATGAAAGTGTGCGCATGATGACCACCGATATGGCAGCGTGT
GCCGGTCTCGGTTATCGGGGAAGAAGTGGCTGATCTCAGCCACCGCGAAAATGACATCAA
AAACGCCATTAACTGATGTTCTGGGGAATATAAATGTCAGGCTCCGTTATACACAGCCA
GTCTGCAGGTGACCATAGTGACTGGATATGTTGTGTTTTACAGTATTATGTAGTCTGTT
TTTTATGCAAAATCTAATTTAATATATTGATATTTATATCATTTCAGTTTCTCGTTCAG
CTTTCTTGTAACAAAGTGGTGATGGGCGGCCGCTCTAGAGGGCCCAAGCTTACGCGTGCA
GCGACGTCATAGCTCTCTCCCTATAGTGAGTCGTATTATAAGCTAGGCACTGGCCGTCGT
TTTACAACGTCGTGACTGGGAAAACGCTAGCTTGGGATCTTTGTGAAGGAACCTTACTT
CTGTGGTGTGACATAATTGGACAAACTACCTACAGAGATTTAAAGCTCTAAGGTAAATAT
AAAATTTTTAAGTGTATAATGTGTTAAACTAGCTGCATATGCTTGCTGCTTGAGAGTTTT
GCTTACTGAGTATGATTTATGAAAATATTATACACAGGAGCTAGTGATTCTAATTGTTTTG
TGTATTTTAGATTACAGTCCCAAGGCTCATTTACAGCCCCCTCAGTCCTCACAGTCTGTT
CATGATCATAATCAGCCATACCACATTTGTAGAGGTTTTACTTGCTTTAAAAAACCTCCC
ACACCTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTTGTTGTTAACTTGTTTAT
TGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTACAAATAAAGCATT
TTTTCTACTGCATTAGTTGTGGTTTTGTCCAACTCATCAATGTATCTTATCATGTCTG
GATCGATCCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTTGCGTATTGGCT
GGCGTAATAGCGAAGAGGCCCCGACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATG
GCGAATGGGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCA
GCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTTCGCTTTCTTCCCTTCT
TTCTCGCCACGTTTCGCGGGCTTTCCCGCTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGT-

FIGURE 91B

TCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAACTTGATTAGGGTGATGGTTCAC
GTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCT
TTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTT
TTGATTTATAAGGGATTTTGCCGATTTCCGGCTATTGGTTAAAAAATGAGCTGATTTAAC
AAATATTTAACGCGAATTTTAACAAAATATTAACGTTTACAATTTTCGCCTGATGCGGTAT
TTTCTCCTTACGCATCTGTGCGGTATTTACACCCGCATACGCGGATCTGCGCAGCACCAT
GGCCTGAAATAACCTCTGAAAGAGGAACTTGGTTAGGTACCTTCTGAGGCGGAAAGAACC
AGCTGTGGAATGTGTGTGTCAGTTAGGGTGTGGAAGTCCCAGGCTCCCAGCAGGCAGAA
GTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAGTCCCAGGCTCCC
CAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCCCCCC
TAACTCCGCCCATCCCGCCCCCTAACTCCGCCCAGTTCGCCCATTTCTCGCCCCCATGGCT
GACTAATTTTTTTTTATTATGTCAGAGGCCGAGGCCGCTCGGCCTCTGAGCTATTCCAGA
AGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTTGATTCTTCTGACA
CAACAGTCTCGAACTTAAGACCATGGCCAAGCCTTTGTCTCAAGAAGAATCCACCCTCAT
TGAAAGAGCAACGGCTACAATCAACAGCATCCCCATCTCTGAAGACTACAGCGTCGCCAG
CGCAGCTCTCTTAGCGACGGCCGCATCTTCACTGGTGTCAATGTATATCATTTTACTGG
GGACCTTGTGCAGAACTCGTGGTGTCTGGGCATGCTGCTGCTGCGGCAGCTGGCAACCT
GACTTGTATCGTCGCGATCGGAAATGAGAACAGGGGCATCTTGAGCCCCCTGCGGACGGTG
CCGACAGGTGCTTCTCGATCTGCATCCTGGGATCAAAGCCATAGTGAAGGACAGTGATGG
ACAGCCGACGGCAGTTGGGATTCTGTGAATTGCTGCCCTCTGGTTATGTGTGGGAGGGCTA
AGCACTTCGTGGCCGAGTTCGAAATGACCGACCAAGCGACGCCCAACCTGCCATCACGAT
GGCCGCAATAAAATATCTTTATTTTATTACATCTGTGTGTGGTTTTTGTGTGAATCG
ATAGCGATAAGGATCCGCGTATGGTGCATCTCAGTACAATCTGCTCTGATGCCGCATAG
TTAAGCCAGCCCCGACACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTC
CCGGCATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTGAGAGGTTT
TCACCGTCATCACCGAAACGCGCGAGACGAAAGGGCCTCGTGATACGCCATTTTTTATAG
GTTAATGTGATGATAATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTG
CGCGGAACCCCTATTTGTTTTATTTTCTAAATACATTCAAATATGTATCCGCTCATGAGA
CAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCACAT
TTCCGTGTGCGCCTTATTCCTTTTTTTCGGGCATTTTGCCTTCTGTTTTTGTCTACCCA
GAAACGCTGGTGAAGTAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTGATC
GAACCTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTTCGCCCCGAAGAACGTTTTTCCA
ATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGG
CAAGAGCAACTCGGTGCGCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTACCA
GTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATA
ACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAG
CTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACCTCGCCTTGATCGTTGGGAACCG
GAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCA
ACAACGTTGCGCAACACTATTAACCTGGCCTAACTTACTCTAGCTTCCCGGCAACAATTA
ATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCT
GGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCA
GCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAG
GCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCAT
TGGTAACTGTGACACCAAGTTTACTCATATATACTTTAGATTGATTTAAACTTTCATTTT
TAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAATCCCTTAA
CGTGAGTTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGA
GATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAACCACCGCTACCAGCG
GTGGTTTGTGTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAAGTGGCTTCAGC
AGAGCGCAGATACCAAATACTGTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAG
AACTCTGTAGACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCC
AGTGCGGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCG
CAGCGGTGCGGCTGAACGGGGGGTTCGTGCACACAGCCAGCTTGGAGCGAACGACCTAC
ACCGAACTGAGATACCTACAGCGTGAGCATTTGAGAAAGCGCCACGCTTCCCGAAGGGAGA
AAGGCGGACAGGTATCCGGTAAGCGGCAGGTCGGAACAGGAGCGCACGAGGAGCTT
CCAGGGGGAAACGCCTGGTATCTTTATAGTCTGTGCGGTTTTCGCCACCTCTGACTTGAG
CGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCG
GCCTTTTTTACGGTTCCTGGCCTTTTGTGCTGACATGTTCTTCTGCGTTA
TCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGC-

FIGURE 91C

AGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGC
AAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGAGCTTGCAATTCGCGCGTT
TTTCAATATTATTGAAGCATTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAA
TGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTCCCCGAAAAGTGCCACCT
GACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTAGTACGAGG
CCCTTTCCTCATTAG

FIGURE 91D

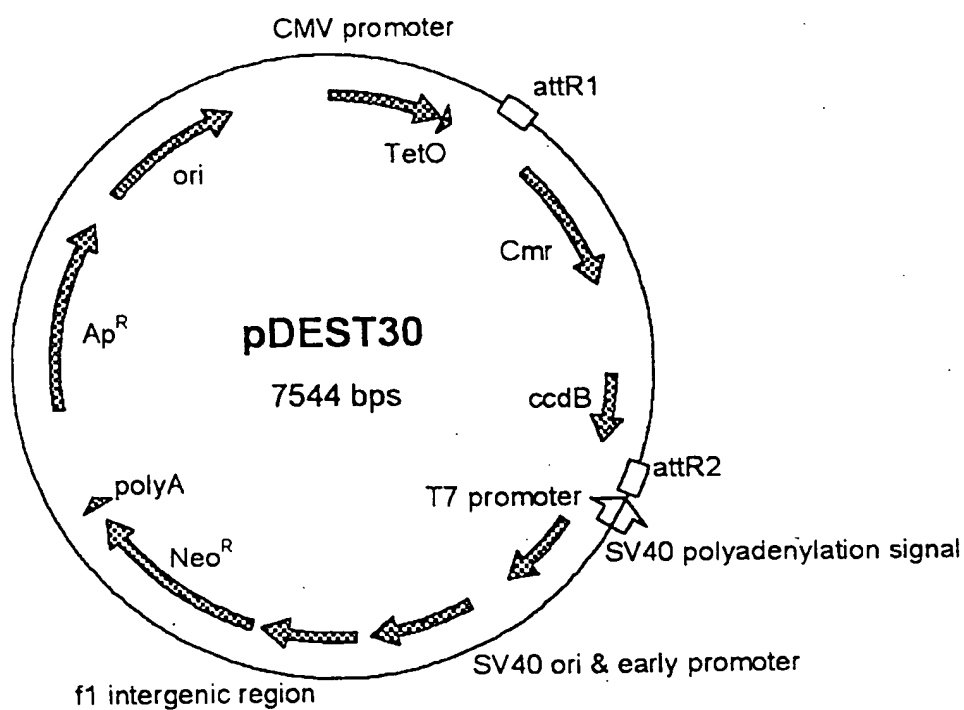


FIGURE 92A

pDEST30 7544 bp

ATGCATGTCGTTACATAACTTACGGTAAATGGCCCCGCTGGCTGACCGCCCAACGACCCC
CGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCAT
TGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTAT
CATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCCGCTGGCATTAT
GCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATC
GCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGAC
TCACGGGGATTTCGAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAA
AATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGT
AGGCGTGACGGTGGGAGGTCTATATAAGCAGAGCTCTCCCTATCAGTGATAGAGATCTC
CCTATCAGTGATAGAGATCGTCGACGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGA
CGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCCAGCCTCCGGACT
CTAGAGGATCCCTACCGGTGATATCCTCGAGCCCATCAACAAGTTTGTACAAAAAGCTG
AACGAGAAAACGTAAATGATATAAATATCAATATATTAATTAGATTTTGCATAAAAAAC
AGACTACATAATACTGTAAAACACAACATATCCAGTCACTATGGCGGCCGATTAGGCAC
CCCAGGCTTTACACTTTATGCTTCCGGCTCGTATAATGTGTGGATTTTGTAGTTAGGATCC
GGCGAGATTTTTCAGGAGCTAAGGAAGCTAAAATGGAGAAAAAATCACTGGATATACCAC
CGTTGATATATCCCAATGGCATCGTAAAGAACATTTTGTAGGCATTTTCAGTCAGTTGCTCA
ATGTACCTATAACAGACCGTTTCAGCTGGATATTACGGCCTTTTTTAAAGACCGTAAAGAA
AAATAAGCACAAAGTTTTATCCGGCCTTTATTTCACATTTCTTGCCCGCCTGATGAATGCTCA
TCCGGAATTCGATGGCAATGAAAGACGGTGAGCTGGTGATATGGGATAGTGTTCACCC
TTGTTACACCGTTTTCCATGAGCAAACGTAACGTTTTTCATCGCTCTGGAGTGAATACCA
CGACGATTTCCGGCAGTTTTCTACACATATATTCGCAAGATGTGGCGTGTACGGTGAAAA
CCTGGCCTATTTCCCTAAAGGGTTTTATTGAGAATATGTTTTTCGTCTCAGCCAATCCCTG
GGTGAGTTTACCAGTTTTGATTTAAACGTGGCCAATATGGACAACCTTCTTCGCCCCCGT
TTTACCATGGGCAATATTATACGCAAGGCGACAAAGGTGCTGATGCCGCTGGCGATTCA
GTACTGCGATGAGTGGCAGGGCGGGCGTAAAGATCTGGATCCGGCTTACTAAAAGCCAG
ATAACAGTATGCGTATTTGCGCGCTGATTTTTTGGCGGTATAAGAATATATACTGATATGTA
TACCCGAAGTATGTCAAAAAGAGGTGTGCTATGAAGCAGCGTATTACAGTGACAGTTGAC
AGCGACAGCTATCAGTTGCTCAAGGCATATATGATGTCAATATCTCCGGTCTGGTAAGCA
CAACCATGCAGAATGAAGCCCCGTCGTCTGCGTGCCGAACGCTGGAAAGCGGAAATCAGG
AAGGGATGGCTGAGGTGCCCCGTTTTATTGAAATGAACGGCTCTTTTGCTGACGAGAACA
GGGACTGGTGAAATGCAGTTTAAGGTTTACACCTATAAAAAGAGAGAGCCGTTATCGTCTG
TTTGTGGATGTACAGAGTGATATTATTGACACGCCCCGGGCGACGGATGGTGATCCCCCTG
GCCAGTGACGCTCTGCTGTGAGATAAAGTCTCCCGTGAACTTTACCCGGTGGTGATATC
GGGGATGAAAGCTGGCGCATGATGACCACCGATATGGCCAGTGTGCCGGTCTCCGTTATC
GGGGAAGAAGTGGCTGATCTCAGCCACCGCGAAAATGACATCAAAAACGCCATTAACCTG
ATGTTCTGGGGAATATAAATGTGAGGCTCCCTTATACACAGCCAGTCTGCAGGTGACCA
TAGTGACTGGATATGTTGTGTTTTACAGTATTATGTAGTCTGTTTTTTATGCAAAATCTA
ATTTAATATATTGATATTTATATCATTTTACGTTTTCTCGTTTCAGCTTTCTTGACAAAGT
GGTTGATGGGCGGCCGCTCTAGAGGGCCCAAGCTTACGCGTGCATGCGACGTCATAGCTC
TCTCCCTATAGTGAGTCGTATTATAAGCTAGGCACTGGCCGTCGTTTTACAACGTCGTGA
CTGGGAAAACCTGCTAGCTTGGGATCTTTGTGAAGGAACCTTACTTCTGTGGTGTGACATA
ATTGGACAAACTACCTACAGAGATTTAAAGCTCTAAGGTAAATATAAAAATTTTAAAGTGT
ATAATGTGTTAAACTAGCTGCATATGCTTGCTGCTTGAGAGTTTTGCTTACTGAGTATGA
TTTATGAAAATATTATACACAGGAGCTAGTGATTCTAATTGTTTGTGATTTTATAGATTCA
CAGTCCCAAGGCTCATTTTCAGGCCCCCTCAGTCCCTCACAGTCTGTTTCATGATCATAATCAG
CCATACCACATTTGTAGAGGTTTTACTTGGCTTTAAAAAACCTCCACACCTCCCCCTGAA
CCTGAAAACATAAAATGAATGCAATTGTTGTTGTTAACTTGTATTGTCAGCTTATAATGG
TTACAAATAAAGCAATAGCATCACAAATTTACAAATAAAGCATTTTTTTCACTGCATTC
TAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCATGTCTGGATCGATCCTGCATT
AATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTTGCGTATTGGCTGGCGTAATAGCGAAG
AGGCCCCGACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGGACGCGC
CCTGTAGCGGCGCATTAAGCGCGGGGTGTGGTTACGCGCAGCGTGACCGTACAC
TTGCCAGCGCCCTAGCGCCCGCTCCTTTTCGCTTTTCCCTTCCTTTCTCGCCACGTTCCG
CCGGCTTTCCCGCTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTT-

FIGURE 92B

TACGGCACCTCGACCCCCAAAAAAGTTGATTAGGGTGATGGTTACGTAAGTGGGCCATCGC
CCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCT
TGTTCCAAAGTGGAAACAACACTCAACCCTATCTCGGTCTATTCTTTTGATTATAAGGGA
TTTTGCCGATTTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAATATTTAACGCGA
ATTTTAACAAATATTTAACGTTTACAATTTTCGCTGATGCGGTATTTCTCCTTACGCAT
CTGTGCGGTATTTACACCCGCATACGCGGATCTGCGCAGCACCATGGCCTGAAATAACCT
CTGAAAGAGGAACCTTGGTTAGGTACCTTCTGAGGCGGAAAGAACAGCTGTGGAATGTGT
GTCAGTTAGGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGC
ATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTA
TGCAAAGCATGCATCTCAATTAGTCAGCAACCATAAGTCCCGCCCTAACTCCGCCCATCC
CGCCCCCTAACTCCGCCAGTTCCGCCCATTTCTCGCCCCATGGCTGACTAATTTTTTTTA
TTTATGCAGAGGCCGAGGCCGCTCGGCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCT
TTTTTGAGGGCCTAGGCTTTTGCAAAAAGCTTGATTCTTCTGACACAACAGTCTCGAAGT
TAAGGCTAGAGCCACCATGATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTG
GGTGGAGAGGCTATTCCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGC
CGTGTTCCGGCTGTCAGCGCAGGGGCGCCCGTTCTTTTTGTCAAGACCGACCTGTCCGG
TGCCCTGAATGAACTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCT
TCCTTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGG
CGAAGTGCCGGGGCAGGATCTCCTGTCTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCAT
CATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATTCGACCA
CCAAGCGAAACATCGCATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTGTCTGATCA
GGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCCGCCAGGCTCAA
GGCGCGCATGCCCGACGGCGAGGATCTCGTCTGACCCATGGCGATGCCTGCTTGCCGAA
TATCATGGTGGAAAATGGCCGCTTTTCTGGATTTCATCGACTGTGGCCGGCTGGGTGTGGC
GGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGCGA
ATGGGCTGACCGCTTCTCGTCTTACGGTATCGCCGCTCCCGATTTCGAGCGCATCGC
CTTCTATCGCCTTCTTGACGAGTTCTTCTGAGCGGGACTCTGGGGTTGAAATGACCGAC
CAAGCGACGCCCAACCTGCCATCACGATGGCCGCAATAAAATATCTTTATTTTCATTACA
TCTGTGTGTTGGTTTTTTGTGTGAATCGATAGCGATAAGGATCCGCGTATGGTGCACCTCT
CAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGCCCCGACACCCGCCAACACCCGC
TGACCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAGACAAGCTGTGACCGT
CTCCGGGAGCTGCATGTGTGAGAGTTTTACCGTCATCACCGAAACGCGCGAGGAAA
GGGCTCGTGATACGCTATTTTTATAGGTTAATGTGATGATAATAATGGTTTCTTAGAC
GTCAGGTGGCACTTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTCTAAAT
ACATTCAAATATGTATCCGCTCATGAGACAATAACCTGATAAATGCTTCAATAATATTG
AAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTGCGCCCTTATCCCTTTTTTGCGGC
ATTTTGCCCTTCTGTTTTTGTCTACCCAGAAACGCTGGTGAAGTAAAAGATGCTGAAGA
TCAGTTGGGTGCACGAGTGGGTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGA
GAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTG
CGCGGTATTATCCCGTATTGACCGCGGCAAGAGCAACTCGGTGCGCCGATACACTATTTC
TCAGAAATGACTTGGTTGAGTACTCACAGTACAGAAAAGCATCTTACGGATGGCATGAC
AGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAAAGTGGGCAACTTACT
TCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCA
TGTAAGTGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCG
TGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAAGTGGGCAACT
ACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGG
ACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGG
TGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTAT
CGTAGTTATCTACAGCAGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGC
TGAGATAGGTGCCTCACTGATTAAGCATTTGGTAAGTGTGAGACCAAGTTTACTCATATAT
ACTTTAGATTGATTTAAACTTCATTTTAAATTTAAAGGATCTAGGTGAAGATCCTTTT
TGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCTGTTCCACTGAGCGTCAGACCC
CGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTT
GCAAAACAAAAAACCACCGCTACAGCGGTGGTTTGTGTTGCCGATCAAGAGCTACCAAC
TCTTTTTCCGAAGGTAAGTGGCTTACAGCGCAGATACCAAAATCTGCTCTTAGT
GTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCT
GCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGA
CTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTGCGGCTGAACGGGGGTTCTGTCAC-

FIGURE 92C

ACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCATTG
AGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGT
CGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCC
TGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCG
GAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCC
TTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGC
CTTTGAGTGAGCTGATAACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAG
CGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCA
TTAATGCAGAGCTTGCAATTGCGCGCTTTTTCAATATTATTGAAGCATTATCAGGGTTA
TTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTC
GCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATCATGACATT
AACCTATAAAAATAGGCGTAGTACGAGGCCCTTTCATCATTAG

FIGURE 92D

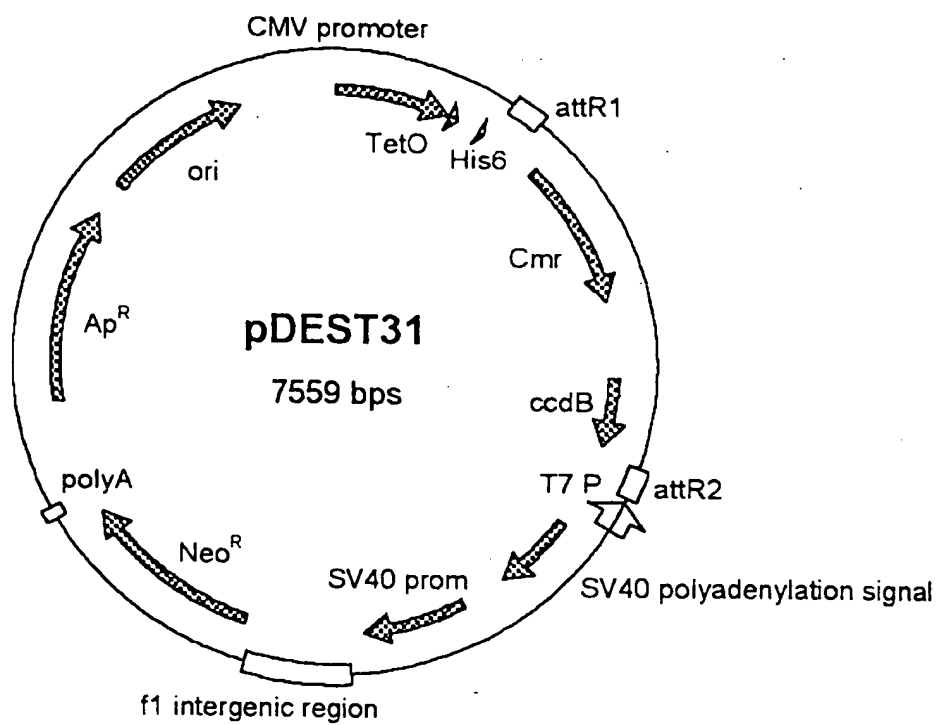


FIGURE 93A

214/240

pDEST31 7559 bp

ATGCATGTCGTTACATAA CTTACGGTAAATGGCCCCGCTGGCTGACCGCCCAACGACCCC
CGCCCATTTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCAT
TGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTAT
CATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCCGCTGGCATTAT
GCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATC
GCTATTACCATGGTGATGCGGTTTTTGGCAGTACATCAATGGGCGTGATAGCGGTTTTGAC
TCACGGGGATTTCGAAGTCTCCACCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAA
AATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGT
AGGCGTGACGGTGGGAGGTCTATATAAGCAGAGCTCTCCCTATCAGTGATAGAGATCTC
CCTATCAGTGATAGAGATCGTCGACGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGA
CGCCATCCACGCTGTTTTGACCTCCATAGAGAACACCGGGACCGATCCAGCCTCCGGACC
ATGGCGTACTACCATCACCATCACCATCACACCGGTGATATCCTCGAGCCCATCACAAGT
TTGTACAAAAAAGCTGAACGAGAAACGTAAAATGATATAAATATCAATATATTAAATTAG
ATTTTGCATAAAAAACAGACTACATAA TACTGTAAAACACAACATATCCAGTCACTATGG
CGGCCGATTAGGCACCCAGGCTTTTACACTTTATGCTTCCGGCTCGTATAATGTGTGGA
TTTTGAGTTAGGATCCGGCGAGATTTTCAGGAGCTAAGGAAGCTAAAATGGAGAAAAAA
TCACTGGATATACCACCGTTGATATATCCCAATGGCATCGTAAAGAACATTTTGAGGCAT
TTCAGTCAGTTGCTCAATGTACCTATAACAGACCGTTTCAGCTGGATATTACGGCCTTTT
TAAAGACCGTAAAGAAAAATAAGCACAAGTTTTATCCGGCCTTTATTACATTCTTGCCC
GCTGATGAATGCTCATCCGGAATTCGCTATGGCAATGAAAGACGGTGAGCTGGTGATAT
GGGATAGTGTTTACCCTTGTTACACCGTTTTCCATGAGCAAACCTGAAACGTTTTTCATCGC
TCTGGAGTGAATACCACGACGATTTCCGGCAGTTTCTACACATATATTCGCAAGATGTGG
CGTGTTACGGTGAAAACCTGGCCTATTTCCCTAAAGGGTTTATTGAGAATATGTTTTTCG
TCTCAGCCAATCCCTGGGTGAGTTTACCAGTTTGTATTAAACGTGGCCAATATGGACA
ACTTCTTCGCCCCCGTTTTTACCATGGGCAAAATATTATACGCAAGGCGACAAGGTGCTGA
TGCCGCTGGCGATTTCAGGTTTCATCATGCCGTCTGTGATGGCTTCCATGTCGGCAGAATGC
TTAATGAATTACAACAGTACTGCGATGAGTGGCAGGGCGGGGCGTAAACGCGTGGATCCG
GCTTACTAAAAGCCAGATAACAGTATGCGTATTTGCGCGCTGATTTTTCGGGTATAAGAA
TATATACTGATATGTATACCCGAAGTATGTCAAAAAGAGGTGTGCTATGAAGCAGCGTAT
TACAGTGACAGTTGACAGCGACAGCTATCAGTTGCTCAAGGCATATATGATGTCAATATC
TCCGGTCTGGTAAGCACAACCATGCAGAATGAAGCCCGTCGTCTGCGTGCCGAACGCTGG
AAAGCGGAAAATCAGGAAGGGATGGCTGAGGTGCGCCGGTTTTATTGAAATGAACGGCTCT
TTTGCTGACGAGAACAGGGACTGGTGAAATGCAGTTTAAAGTTTTACACCTATAAAAGAGA
GAGCCGTTATCGTCTGTTTTGTGGATGTACAGAGTGATATTATTGACACGCCCCGGCGACG
GATGGTGATCCCCCTGGCCAGTGCACGTCTGCTGTCAGATAAAGTCTCCCGTGAACCTTTA
CCCCGTGGTGATATCGGGGATGAAAGCTGGCGCATGATGACCACCGATATGGCCAGTGT
GCCGGTCTCCGTTATCGGGGAAGAAGTGGCTGATCTCAGCCACCGCGAAAATGACATCAA
AAACGCCATTAACTGATGTTCTGGGGAATATAAATGTGAGGCTCCGTTATACACAGCCA
GTCTGCAGGTCGACCATAGTGACTGGATATGTTGTGTTTTACAGTATTATGTAGTCTGTT
TTTTATGCAAAATCTAATTTAATATATTGATATTTATATCATTTTACGTTTCTCGTTTACG
CTTCTTGTAACAAGTGGTGATGGGCGGCCGCTCTAGAGGGCCCAAGCTTACGCGTGAT
GCGACGTCATAGCTCTCTCCCTATAGTGAGTCGTATTATAAGCTAGGCACTGGCCGTGCT
TTTACAACGTCGTGACTGGGAAAACCTGCTAGCTTGGGATCTTTGTGAAGGAACCTTACTT
CTGTGGTGTGACATAATTGGACAAACTACCTACAGAGATTTAAAGCTCTAAGGTAAATAT
AAAATTTTTAAGTGATATAATGTGTTAAACTAGCTGCATATGCTTGTGCTTGTGAGATTTT
GCTTACTGAGTATGATTTATGAAAATATTATACACAGGAGCTAGTGATTCTAATTGTTTG
TGATTTTATAGATTCACAGTCCCAAGGCTCATTTTACGGCCCCCTCAGTCCTCACAGTCTGTT
CATGATCATAATCAGCCATACCAATTTGTAGAGGTTTTACTTGCTTTAAAAAACCTCCC
ACACCTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTTGTTGTTAACTTGTTTAT
TGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTACAAATAAAGCATT
TTTTTCACTGCATTCTAGTTGTGTTTTGTCCAACTCATCAATGTATCTTATCATGTCTG
GATCGATCCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGGCTATTGGCT
GGCGTAATAGCGAAGAGGCCCCGACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATG
GCGAATGGGACGCGCCCTGTAGCGGCGCATTAAAGCGCGGCGGTTGTTGTTTACGCGCA
GCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTTCGCTTTCTTCCCTTCTT
TTCTCGCCACGTTTCGCCGGCTTTCCCGCTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGT-

Figure 93B

TCCGATTTAGTGCTTTACGGCACCTCGACCCCCAAAAAAGCTTGATTAGGGTGATGGTTTAC
GTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCT
TTAATAGTGGACTCTGTTCCTAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTT
TTGATTTTATAAGGGATTTTGCCGATTTTCGCCCTATTGGTTAAAAAATGAGCTGATTTAAC
AAATATTTTAAACGCGAATTTTAAACAAAATATTAACGTTTACAATTTTCGCTGATGCGGTAT
TTTCTCCTTACGCATCTGTGCGGTATTTACACCCGCATACGCGGATCTGCGCAGCACCAT
GGCCTGAAATAACCTCTGAAAGAGGAAGTTGGTTAGGTACCTTCTGAGGCGGAAAGAACC
AGCTGTGGAATGTGTGTGAGTTAGGGTGTGGAAGTCCCCAGGCTCCCCAGCAGGCAGAA
GTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAGTCCCCAGGCTCCC
CAGCAGGCAGAAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCCGCCCC
TAACTCCGCCCCCTCCCGCCCCCTAACTCCGCCCCAGTTCCGCCCCATTCTCCGCCCCATGGCT
GACTAATTTTTTTTATTTTATGTCAGAGGCCGAGGCCCTCGGCCTCTGAGCTATTCCAGA
AGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTTGATTCTTCTGACA
CAACAGTCTCGAACTTAAGGCTAGAGCCACCATGATTGAACAAGATGGATTGCACGCAGG
TTCTCCGGCCGCTTGGGTGGAGAGGCTATTTCGGCTATGACTGGGCACAACAGACAATCGG
CTGCTCTGATGCCGCCGTGTTCGGCTGTGAGCGCAGGGGCGCCCGGTTCTTTTTGTCAA
GACCGACCTGTCCGGTGCCCTGAATGAAGTGCAGGACGAGGCAGCGCGGCTATCGTGGCT
GGCCACGACGGGCGTTCTTGGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGA
CTGGCTGCTATTGGGCGAAGTGCCGGGCGAGGATCTCTGTCTCATCTCACCTTGCTGCG
CGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCTAC
CTGCCCCATTGACACCAAGCGAAACATCGCATCGAGCGAGCACGTAAGTCCGGATGGAAGC
CGGTCTTGTGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAAGT
GTTCCGCCAGGCTCAAGGCGCGCATGCCCGACGGCGAGGATCTCGTCTGACCCATGGCGA
TGCCTGCTTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATTCTGACTGTGG
CCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGA
AGAGCTTGCGGCGCAATGGGCTGACCGCTTCTCTGCTGCTTTACGGTATCGCCGCTCCCGA
TTCCGACGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAGCGGGACTCTGGGG
TTTCAAAATGACCGACCAAGCGACGCCAACCTGCCATCACGATGGCCGCAATAAAATATC
TTTATTTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATCGATAGCGATAAGGATCCG
CGTATGGTGCATCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGCCCCGACA
CCCCCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAG
ACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTGAGAGTTTTTACCGTCATCACCGAA
ACGCGCGAGACGAAAGGGCTCTGTGATACGCCCTATTTTATAGGTTAATGTGATGATAAT
AATGGTTTTCTTAGCGTCAAGTGGCACTTTTTCGGGGAATGTGCGCGGAACCCCTATTTG
TTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCGTGATAAAT
GCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTGCGCCCTTAT
TCCCTTTTTTGGCGCATTTTGCCTTCTGTTTTTGTCTACCCAGAAACGCTGGTGAAAGT
AAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTACATCGAACTGGATCTCAACAG
CGGTAAGATCCTTGAGAGTTTTTCGCCCCGAAGAAGCTTTTCCAATGATGAGCACTTTTAA
AGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTGCG
CCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTACCAGTCACAGAAAAGCATCT
TACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACAC
TGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGTCA
CAACATGGGGGATCATGTAAGTGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCAT
ACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACT
ATTAAGTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGC
GGATAAAGTTGACGAGCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTTATTGCTGA
TAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGG
TAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACG
AATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAAGTGTGAGACCA
AGTTTACTCATATATACTTTAGATTGATTTAAACTTCAATTTTAAATTTAAAGGATCTA
GGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTTCGTTCCA
CTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCG
CGTAATCTGCTGCTTGCAAAACAAAAAACCACCGCTACCAGCGGTGGTTTTGTTTGGCGGA
TCAAGAGCTACCAACTCTTTTTCCGAAGGTAAGTGGCTTACGAGAGCGCAGATACCAAA
TACTGTCTCTGTAGCGTAGCTTAGTGGCCACCACTTCAAGAACTCTGTAGCAGCGCC
TACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTG
TCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTGCGGCTGAAC-

Figure 93C

GGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCT
ACAGCGTGAGCATTGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCC
GGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTG
GTATCTTTATAGTCCTGTCTGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATG
CTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCT
GGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGA
TAACCGTATTACCGCCTTTGAGTGAGCTGATAACCGCTCGCCGCAGCCGAACGACCGAGCG
CAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGC
GCGTTGGCCGATTCATTAATGCAGAGCTTGCAATTCGCGCGTTTTTCAATATTATTGAAG
CATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAA
ACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCAT
TATTATCATGACATTAACCTATAAAAATAGGCGTAGTACGAGGCCCTTCACTCATTAG

217/240

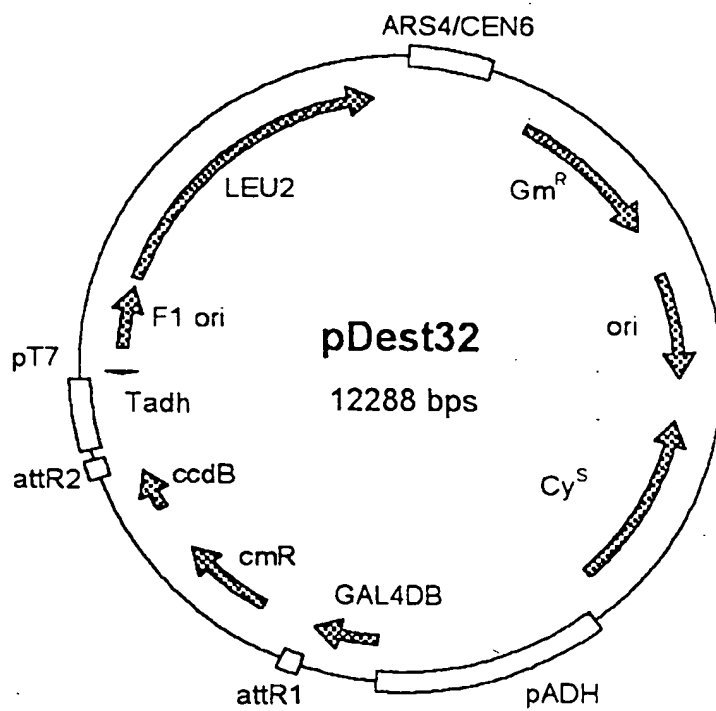


FIGURE 94A

pDEST32 12288 bp

GACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCATGATAATAATGGTTT
CTTAGGACGGATCGCTTGCTGTAACCTACACGCGCCTCGTATCTTTAATGATGGAATA
ATTTGGGAATTTACTCTGTGTTTATTTATTTTTATGTTTTGTATTTGGATTTTAGAAAGT
AAATAAAGAAGGTAGAAGAGTTACGGAATGAAGAAAAAATAAACAAAGGTTTAAAAA
ATTTCAACAAAAAGCGTACTTTACATATATATTTATTAGACAAGAAAGCAGATTAAATA
GATATACATTCGATTAACGATAAGTAAATGTAAATCACAGGATTTTCGTGTGTGGTCT
TCTACACAGACAAGATGAAACAATTCGGCATTAAATACCTGAGAGCAGGAAGAGCAAGATA
AAAGGTAGTATTTGTTGGCGATCCCCCTAGAGTCTTTTACATCTTCGGAAAAACAAAACT
ATTTTTCTTTAATTTCTTTTCTTTTACTTTCTATTTTAAATTTATATATTTATATTAAAAA
ATTTAAATTATAATTATTTTATAGCACGTGATGAAAAGGACCCAGGTGGCACTTTTCGG
GGAAATGTGCGCGGAACCCCTATTTGTTTATTTTCTAAATACATTCAAATATGTATCCG
CTCATGAGACAATAACCTGATAAATGCTTCAATAATCTGCAGTGCAGGCCCCGTGTC
TCAAAATCTCTGATGTTACATTGCACAAGATAAAAAATATATCATCATGAACAATAAACT
GTCTGCTTACATAAACAGTAATAACAAGGGGTGTTATGAGCCATATTCAACGGGAACGTC
TTGCTGGAGGCCGCGATTAAATTCACATGGATGCTGATTTATATGGGTATAAATGGGC
TCGGTAGCCAACCACTAGAACTATAGCTAGAGTCCTGGGCGAACAACGATGCTCGCCTT
CCAGAAAACCCAGGATGCGAACCACTTCATCCGGGGTCAGCACACCAGCGCAAGCGCCGCG
ACGGCCGAGGTCTTCCGATCTCCTGAAGCCAGGGCAGATCCGTGCACAGCACCTTGCCGT
AGAAGAACAGCAAGGCCGCCAATGCCTGACGATGCGTGGAGACCGAAACCTTGCGCTCGT
TCGCCAGCCAGGACAGAAATGCCTCGACTTCGCTGCTGCCAAGGTTGCCGGGTGACGCA
CACCGTGGAAACGGATGAAGGCACGAACCCAGTTGACATAAGCCTGTTCCGTTTCGTAAAC
TGTAATGCAAGTAGCGTATGCGCTCACGCACTGGTCCAGAACCTTGACCGAACGCAGCG
GTGGTAACGGCGCAGTGGCGGTTTTCATGGCTTGTATGACTGTTTTTTGTACAGTCTA
TGCCTCGGGCATCCAAGCAGCAAGCGCTTACGCCGTGGGTGATGTTTGATGTTATGGA
GCAGCAACGATGTTACGCAGCAGCAACGATGTTACGCAGCAGGGCAGTCGCCCTAAAACA
AAGTTAGGTGGCTCAAGTATGGGCATCATTGCACATGTAGGCTCGGCCCTGACCAAGTC
AAATCCATGCGGGCTGCTCTTGATCTTTTCGGTCTGTAGTTCCGAGACGTAGCCACCTAC
TCCCAACATCAGCCGGACTCCGATTACCTCGGGAACCTTGCTCCGTAGTAAGACATTCATC
GCGCTTGCTGCCTTCGACCAAGAAGCGGTTGTTGGCGCTCTCGCGGCTTACGTTCTGCCC
AGGTTTGAGCAGCCGCGTAGTGAGATCTATATCTATGATCTCGCAGTCTCCGGCGAGCAC
CGGAGGCAGGGCATTGCCACCGCGCTCATATCTCTCAAGCATGAGGCCAACCGGCTT
GGTGCTTATGTGATCTACGTGCAAGCAGATTACGGTGACGATCCCGCAGTGGCTCTCTAT
ACAAAGTTGGGCATACGGGAAGAAGTGATGCACTTTGATATCGACCCAAGTACCGCCACC
TAACAATTCGTTCAAGCCGAGATCGGCTTCCCGGCCAATAGGTTGTATTGATGTTGGAC
GAGTCGGAATCGCAGACCGATACAGGATCTTGCCATCCTATGGAACCTGCCTCGGTGAGT
TTTCTCCTTCATTACAGAAACGGCTTTTTCAAAAATATGGTATTGATAATCCTGATATGA
ATAAATTGCAGTTTCATTTGATGCTCGATGAGTTTTTCTAATCAGAATTGGTTAATTTGGT
TGTAACACTGGCAGAGCATTACGCTGACTTGACGGGACGGCGNCATGACCAAAATCCCTT
AACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTT
GAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAG
CGGTGGTTTGTGTTGCCGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACCTGGCTTCA
GCAGAGCGCAGATACCAATACTGTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCA
AGAATCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAAGTGGCTGCTG
CCAGTGGCGATAAGTCGTGCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGG
CGCAGCGGTCCGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGAGCGAACGACCT
ACACCGAACTGAGATACCTACAGCGTGAGCATTGAGAAAGCGCCACGCTTCCCGAAGGGA
GAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGC
TTCCAGGGGGGAACGCCTGGTATCTTTATAGTCTGTGCGGTTTCGCCACCTCTGACTTG
AGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGAGCCTATGGAAAAACGCCAGCAACG
CGGCCCTTTTACGGTTCTTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTCTGCGT
TATCCCTGATTCGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCC
GCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATAC
GCAACCGCCTCTCCCCGCGCGTTGGCCGATTATTAATGCAGCTGGCACGACGAGTTTC
CCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTACCTCACTCATTAGG
CACCCAGGCTTTTACACTTATGCTTCCGGCTCCTATGTTGTGTGGAATTGTGAGCGGAT
AACAAATTCACACAGGAAACAGCTATGACCATGATTACGCCAAGCTCGGAATTAACCTC-

FIGURE 94B

ACTAAAGGGAACAAAAGCTGGTACCGATCCCGAGCTTTGCAAATTAAAGCCTTCGAGCGT
CCCAAAACCTTCTCAAGCAAGGTTTTTCAAGTATAATGTTACATGCGTACACGCGTCTGTAC
AGAAAAAAGAAAAATTTGAAATATAAATAACGTTCTTAATACTAACATAACTATAAAA
AAATAAATAGGGACCTAGACTTCAGGTTGTCTAACTCCTTCCTTTTCGGTTAGAGCGGAT
GTGGGGGGAGGGCGTGAATGTAAGCGTGACATACTAATTACATGATATCGACAAAGGAA
AAGGGGCCTGTTTACTCACAGGCTTTTTTCAAGTAGGTAATTAAGTCGTTTCTGTCTTTT
TCCTTCTTCAACCCACCAAGGCCATCTTGGTACTTTTTTTTTTTTTTTTTTTTTTTTTT
TT
TTTTTTTTCATAGAAATAATACAGAAGTAGATGTTGAATTAGATTAACTGAAGATATAT
AATTTATTGGAAAATACATAGAGCTTTTTTGTGATGCGCTTAAGCGATCAATTCAACAAC
ACCACCAGCAGCTCTGATTTTTTTCTTCAGCCAACCTGGAGACGAATCTAGCTTTGACGAT
AACTGGAACATTTTGGAAATCTACCTTACCCTAACGATCTTACCGTAACCGGCTGCCAAAGT
GTCAATAACTGGAGCAGTTTCTTAGAAGCAGATTTCAAGTATTGGTCTCTCTTGTCTTC
TGGGATCAATGTCCACAATTTGTCCAAGTTCAAGACTGGCTTCCAGAAATGAGCTTGTG
CTTGTGGAAGTATCTCATACCAACCTTACCGAAATAACCTGGATGGTATTTATCCATGTT
AATTCTGTGGTGTGTTGACCACCGGCCATACCTCTACCACCGGGGTGCTTTCTGTGCTT
ACCGATACGACCTTACCAGGCTGAGACGTGACCTCTGTGCTTTCTAGTCTTAGTGAATCT
GGAAGGCATTCTTGATTAGTTGGATGATTGTTCTGGGATTTAATGCAAAAATCACTTAAG
AAGGAAAAATCAACGAGAGAAAGCAACGCCATCTTAAATATACGGGATACAGATGAAAGGG
TTTGAACCTATCTGGAAAATAGCATTAAACAAGCGAAAACTGCGAGGAAAATGTTTGC
GTCTCTGCGGGCTATTACGCGCCAGAGGAAAATAGGAAAATAACAGGGCATTAGAAAA
ATAATTTTGATTTTGGTAATGTGTGGGTCTGGTGTACAGATGTTACATTGGTTACAGTA
CTCTTGTTTTTGCTGTGTTTTTCGATGAATCTCCAAAATGGTTGTTAGCACATGGAAGAG
TCACCGATGCTAAGTTATCTCTATGTAAGCTACGTGGCGTGACTTTTGATGAAGCCGCAC
AAGAGATACAGGATTGGCAACTGCAATAGAATCTGGGGATCCCCCTCGAGATCCGGGA
TCGAAGAAATGATGTTAAATGAAATAGGAAATCAAGGAGCATGAAGGCAAAAAGACAAATA
TAAGGGTCGAACGAAAAAATAAAGTGAAGAGTGTGATATGATGATTTGGCTTTGCGGCG
CCGAAAAAACGAGTTTACGCAATTGCACAATCATGCTGACTCTGTGGCGGACCCGCGCTC
TTGCCCGCCCCGCGATAACGCTGGGCGTGAGGCTGTGCCCGGCGGAGTTTTTTGCGCCTG
CATTTTCCAAGGTTTACCCTGCGCTAAGGGGCGAGATTGGAGAAGCAATAAGAATGCCGG
TTGGGGTTGCGATGATGACGACCACGACAACCTGGTGTCAATTATTTAAGTTGCCGAAAGAA
CCTGAGTGCATTTGCAACATGAGTATACTAGAAGAATGAGCCAAGACTTGCGAGACGGA
GTTTGCCGGTGGTGCGAACAATAGAGCGACCATGACCTTGAAGGTGAGACGCGCATAACC
GCTAGAGTACTTTGAAGAGGAAACAGCAATAGGGTTGCTACCAGTATAAATAGACAGGTA
CATACAACACTGGAAATGGTTGTCTGTTTGAGTACGCTTTCAATTCATTTGGGTGTGCAC
TTTATTATGTTACAATATGGAAGGGAACCTTACACTTCTCCTATGCACATATATTAATTA
AAGTCCAATGCTAGTAGAGAAGGGGGGTAACACCCCTCCGCGCTCTTTTCCGATTTTTTT
CTAAACCGTGAATATTTCCGATATCCTTTTGTGTTTCCGGGTGTACAATATGGACTTC
CTCTTTTCTGGCAACCAACCCATACATCGGGATTCTTATAATACCTTCGTTGGTCTCCC
TAACATGTAGGTGGCGGAGGGGAGATATACAATAGAACAGATACCAGACAAGACATAATG
GGCTAAACAAGACTACACCAATTACACTGCCTCATTGATGGTGGTACATAACGAACTAAT
ACTGTAGCCCTAGACTTGATAGCCATCATCATATCGAAGTTTCACTACCCTTTTCCATT
TGCCATCTATTGAAGTAATAATAGGCGCATGCAACTTCTTTCTTTTTTTTTCTTTTCTC
TCTCCCCGTTGTTGTCTCACCATATCCGCAATGACAAAAAAATGATGGAAGACACTAA
AGGAAAAAATTAACGACAAAGACAGCACCAACAGATGTCGTTGTTCCAGAGCTGATGAGG
GGTATCTTCGAACACACGAAACTTTTTCTTCTTTCATTACGCACACTACTCTCTAATG
AGCAACGGTATACGGCCTTCTTCCAGTTACTTGAATTTGAAATAAAAAAAGTTTGCCGC
TTTGCTATCAAGTATAAATAGACCTGCAATTATTAATCTTTTGTTTCTCGTCATTGTTT
TCGTTCCCTTTCTTCTTGTCTTTTCTGCACAATATTTCAAGCTATACCAAGCATAC
AATCAACTCCAAGCTTGAAGCAAGCCTCCTGAAAGATGAAGCTACTGTCTTCTATCGAAC
AAGCATGCGATATTTGCCGACTTAAAAAGCTCAAGTGCTCCAAAGAAAAACCGAAGTGCG
CCAAGTGTCTGAAGAACAACCTGGGAGTGTGCTACTCTCCCAAAACCAAAAGGTCTCCGC
TGACTAGGGCACATCTGACAGAAGTGAATCAAGGCTAGAAAGACTGGAACAGCTATTTT
TACTGATTTTTCTCGAGAAGACCTTGACATGATTTTGAAAATGGATTCTTTACAGGATA
TAAAGCATTTGTTAACAGGATTATTTGTACAAGATAATGTGAATAAAGATGCCGTACAG
ATAGATTGGCTTCAGTGGAGACTGATATGCCCTTAACATTGAGACAGCATAGAATAAGTG
CGACATCATCATCGGAAGAGAGTAGTAACAAAGGTCAAAGACAGTTGACTGTATCGTCCA
GGTCCAATCAACAAGTTTGTACAAAAAAGCTGAACGAGAAACGTAAATGATATAAATA-

FIGURE 94C

TCAATATATTAAATTAGATTTTGCATAAAAAACAGACTACATAATACTGTAAAAACAAC
ATATCCAGTCACATATGGCGGCGCTAAGTTGGCAGCATACCCGACGCACCTTTGCGCCGA
ATAAATACCTGTGACGGAAGATCACTTCGCAGAATAAATAAATCCTGGTGTCCCTGTTGA
TACCGGGAAGCCCTGGGCCAACTTTTGGCGAAAATGAGACGTTGATCGGCACGTAAGAGG
TTCCAACTTTCACCATAATGAAATAAGATCACTACCGGGCGTATTTTTTGTAGTTATCGAG
ATTTTCAGGAGCTAAGGAAGCTAAAATGGAGAAAAAATCACTGGATATACCACCGTTGA
TATATCCCAATGGCATCGTAAAGAACATTTTGAGGCATTTTCAGTCAGTTGCTCAATGTAC
CTATAACCAGACCGTTTCAGCTGGATATTACGGCCTTTTTTAAAGACCGTAAAGAAAAATAA
GCACAAGTTTTATCCGGCCTTTATTCACATTTCTTGCCCGCCTGATGAATGCTCATCCGGA
ATTCCGTATGGCAATGAAAGACGGTGAGCTGGTGATATGGGATAGTGTTACCCCTTGTTA
CACCGTTTTCCATGAGCAAACTGAAACGTTTTTCATCGCTCTGGAGTGAATACCACGACGA
TTTCCGGCAGTTTCTACACATATATTCGCAAGATGTGGCGTGTTACGGTGAAAACCTGGC
CTATTTCCCTAAAGGGTTTATTGAGAATATGTTTTTCGTCTCAGCCAATCCCTGGGTGAG
TTTCACCAAGTTTTGATTTAAACGTGGCCAATATGGACAACCTTCTTCGCCCCCGTTTTAC
CATGGGCAAATATTATACGCAAGGCGACAAGGTGCTGATGCCGCTGGCGATTTCAGGTTCA
TCATGCCGTCTGTGATGGCTTCCATGTCTGGCAGAATGCTTAATGAATTACAACAGTACTG
CGATGAGTGGCAGGGCGGGCGTAATCTAGAGGATCCGGCTTACTAAAAGCCAGATAACA
GTATGCGTATTTGCGCGCTGATTTTTTGGCGGTATAAGAATATATACTGATATGTATACCCG
AAGTATGTCAAAAAGAGGTGTGCTATGAAGCAGCGTATTACAGTGACAGTTGACAGCGAC
AGCTATCAGTTGCTCAAGGCATATATGATGTCAATATCTCCGGTCTGGTAAGCACAACCA
TGCAGAATGAAGCCCGTCTGCTGCGTGCCGAACGCTGGAAAGCGGAAAATCAGGAAGGGA
TGGCTGAGGTGCGCCCGTATTATTGAAATGAACGGCTCTTTTGCTGACGAGAACAGGGACT
GGTGAAATGCAGTTTAAAGTTTACACCTATAAAAGAGAGAGCCGTTATCGTCTGTTTGTG
GATGTACAGAGTGATATTATTGACACGCGCGGCGACGGATGGTGATCCCCCTGGCCAGT
GCACGCTGCTGTGATGATAAAGTCTCCCGTGAACCTTACCCGGTGGTGATATCGGGGAT
GAAAGCTGGCGCATGATGACCACCGATATGGCCAGTGTGCCGGTCTCCGTTATCGGGGAA
GAAGTGGCTGATCTCAGCCACCGCGAAAATGACATCAAAAACGCCATTAACCTGATGTTT
TGGGGAATATAAATGTCAGGCTCCCTTATACACAGCCAGTCTGCAGGTGACCATAGTGA
CTGGATATGTTGTGTTTTACAGTATTATGTAGTCTGTTTTTTATGCAAAATCTAATTTAA
TATATTGATATTTATATCATTTTACGTTTCTCGTTTCAGCTTTCTTGTAACAAAGTGGTTG
ATGGCCGCTAAGTAAGTAAGACGTCGAGCTCTAAGTAAGTAACGGCCGCCACCGCGGTGG
AGCTTTTGGACTTCTCGCCAGAGGTTTGGTCAAGTCTCCAATCAAGGTTGTCCGGTTGTC
TACCTTGCCAGAAAATTTACGAAAAGATGAAAAGGGTCAAATCGTTGGTAGATACGTTGT
TGACACTTCTAAATAAGCGAATTTCTTATGATTTATGATTTTTATTATTAATAAGTTAT
AAAAAAATAAGTGTATACAAATTTTAAAGTGACTCTTAGGTTTTAAACGAAAATTTCTT
GTTCTTGAGTAACCTCTTCTGTAGGTGAGGTGCTTTCTCAGGTATAGCATGAGGTGCG
TCTTATTGACCACACCTCTACCGGCATGCCGAGCAAATGCCTGCAAAATCGCTCCCCATTT
CACCCAATTGTAGATATGCTAACTCCAGCAATGAGTTGATGAATCTCGGTGTGATTTTA
TGCTCTCAGAGGACAATACCTGTTGTAATCGTTCTTCCACACGGATCCCAATTCGCCCTA
TAGTGAGTCGTATTACAATTCAGTGGCCGTGCTTTTACAACGTCGTGACTGGGAAAAACC
TGGCGTTACCCAACCTTAATCGCCTTGACGACATCCCCCTTTCGCCAGCTGGCGTAATAG
CGAAGAGGCCCCGACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGAC
GCGCCCTGTAGCGGCGCATTAAAGCGCGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCT
ACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTGCTTTCTTCCCTTCTTCTCGCCACG
TTCGCCGGCTTTCGCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGT
GCTTTACGGCACCTCGACCCCAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCA
TCGCCCTGATAGACGGTTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGA
CTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTTTTGATTTATAA
GGGATTTTGCCGATTTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAAC
GCGAATTTTAACAAAATATTAACGTTTACAATTTCTGATGCGGTATTTTCTCCTTACGC
ATCTGTGCGGTATTTACACCGCATATCGACCGGTGAGGAGAACTTCTAGTATATCCAC
ATACCTAATATTATTGCCTTATTAATAATGGAATCGGAACAATTACATCAAAATCCACAT
TCTCTTCAAAATCAATTGTCCTGTACTTCTTGTTCATGTGTGTTCAAAAACGTTATATT
TATAGGATAATTATACTCTATTCTCAACAAGTAATTGGTTGTTTGGCCGAGCGGCTTAA
GGCGCCTGATTCAAGAAATATCTTGACCGCAGTTAACTGTGGGAATACTCAGGTATCGTA
AGATGCAAGAGTTGCAATCTCTTAGCAACCATTATTTTTTCTCAACATAACGAGAACA
CACAGGGGCGCTATCGCACAGAATCAAATTCGATGACTGGAAATTTTTTGTAAATTCAG
AGGTGCGCTGACGCATATACCTTTTTCACTGAAAAATTTGGGAGAAAAAGGAAAGGTGAG-

FIGURE 94D

AGGCCGGAACCGGCTTTTCATATAGAATAGAGAAGCGTTCATGACTAAATGCTTGCATCA
CAATACCTTGAAGTTGACAATATTATTTAAGGACCTATTGTTTTTTCCAATAGGTGGTTAG
CAATCGTCTTACTTTCTAACTTTTCTTACCTTTTACATTTTACGCAATATATATATATATT
TCAAGGATATACCATTTCTAATGTCTGCCCCATGTCTGCCCCCTAAGAAGATCGTCTGTTTT
GCCAGGTGACCACGTTGGTCAAGAAATCACAGCCGAAGCCATTAAGGTTCTTAAAGCTAT
TTCTGATGTTTCTTCCAATGTCAAGTTCGATTTTCAAAAATCATTAAATTGGTGGTGCTGC
TATCGATGCTACAGGTGTCCCACTTCCAGATGAGGCGCTGGAAGCCTCCAAGAAGGTTGA
TGCCGTTTTGTTAGGTGCTGTGGGTGGTCCCTAAATGGGGTACCGGTAGTGTAGACCTGA
ACAAGGTTTTACTAAAAATCCGTAAAGAACTTCAATTGTACGCCAACTTAAGACCATGTAA
CTTTGCATCCGACTCTCTTTTAGACTTATCTCCAATCAAGCCACAATTTGCTAAAGGTAC
TGACTTCGTTGTTGTCAGAGAATTAGTGGGAGGTATTTACTTTGGTAAGAGAAAGGAAGA
CGATGGTGATGGTGTGCTGCTTGGGATAGTGAACAATACACCGTTCAGAAAGTGCAAAGAAT
CACAAGAATGGCCGCTTTTCATGGCCCTACAACATGAGCCACCATTGCCTATTTGGTCTTT
GGATAAAGCTAATGTTTTGGCCTCTTCAAGATTATGGAGAAAACTGTGGAGGAAACCAT
CAAGAACGAATTCCTTACATTGAAGGTTCAACATCAATTGATTGATTCTGCCGCCATGAT
CCTAGTTAAGAACCCCAACCCACCTAAATGGTATTATAATCACCAGCAACATGTTTGGTGA
TATCATCTCCGATGAAGCCTCCGTTATCCAGGTTCTTGGGTTTGTGGCATCTGCGTC
CTTGGCCTCTTTGCCAGACAAGAACACCGCATTTGGTTTGTACGAACCATGCCACGGTTC
TGCTCCAGATTTGCCAAAGAATAAGGTTGACCCTATCGCCACTATCTTGTCTGCTGCAAT
GATGTTGAAATTGTCATTGAACCTTGCCTGAAGAAGGTAAGGCCATTGAAGATGCAGTTAA
AAAGGTTTTGGATGCAGGTATCAGAACTGGTGATTTAGGTGGTTCCAACAGTACCACCGA
AGTCGGTGATGCTGTGCGCGAAGAAGTTAAGAAAATCCTTGCTTAAAAAGATTCTCTTTT
TTTATGATATTTGTACATAAACTTTATAAATGAAATTCATAATAGAAACGACACGAAATT
ACAAAATGGAATATGTTTCATAGGGTAGACGAACTATATACGCAATCTACATACATTTAT
CAAGAAGGAGAAAAAGGAGGATAGTAAAGGAATACAGGTAAGCAAATTGATACTAATGGC
TCAACGTGATAAGGAAAAAGAATTGCACTTTAAACATTAATATTGACAAGGAGGAGGGCAC
CACACAAAAAGTTAGGTGTAACAGAAAATCATGAACTACGATTCCTAATTTGATATTGG
AGGATTTTCTCTAAAAAATAACAACAAATAAAAAACACTCAATGACCTGACCAT
TTGATGGAGTTTAAGTCAATACCTTCTTGAACCATTTCCCATATGGTGAAAGTTCCCTC
AAGAATTTTACTCTGTGAGAAACGGCCTTACGACGTAGTCGATATGGTGCACCTCTCAGTA
CAATCTGCTCTGATGCCGCATAGTTAAGCCAGCCCCGACACCCGCCAACACCCGCTGACG
CGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAGACAAGCTGTGACCGTCTCCG
GGAGCTGCATGTGTGAGAGTTTTACCGTCATCACCGAACGCGCGA

FIGURE 94E

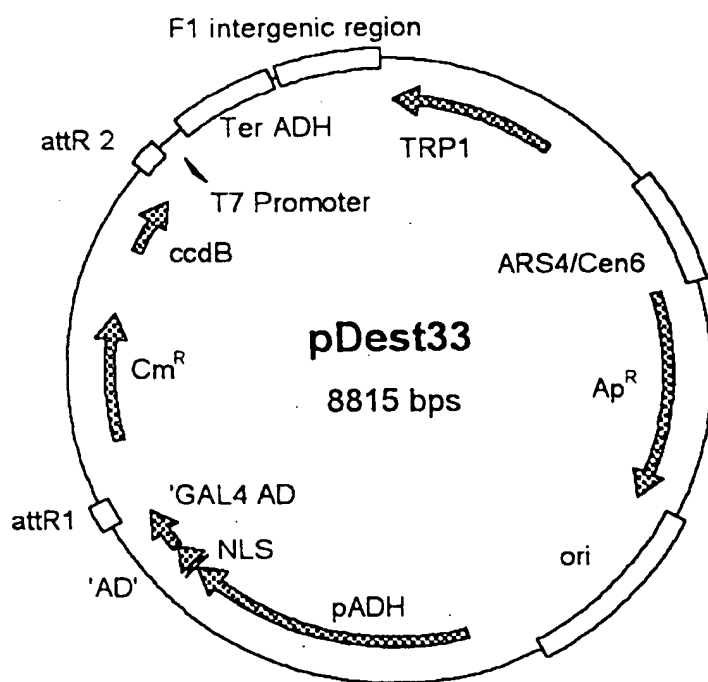


FIGURE 95A

pDEST33 8815 bp

GCCTTACGCATCTGTGCGGTATTTACACCCGAGGCAAGTGACAAAACAATACTTAAATA
AATACTACTCAGTAATAACCTATTTCTTAGCATTTTTTGACGAAATTTGCTATTTTGTAG
AGTCTTTTACACCATTTGTCTCCACACCTCCGCTTACATCAACACCAATAACGCCATTTA
ATCTAAGCGCATCACCAACATTTTCTGGCGTCAGTCCACCAGCTAACATAAAATGTAAGC
TTTCGGGGCTCTCTTGCCCTTCCAACCCAGTCAGAAATCGAGTTCCAATCCAAAAGTTTAC
CTGTCCCACCTGCTTCTGAATCAAACAAGGGAATAAACGAATGAGGTTTCTGTGAAGCTG
CACTGAGTAGTATGTTGCAGTCTTTTGGAAATACGAGTCTTTTAATAACTGGCAAACCGA
GGAACCTCTGGTATTCTTGCCACGACTCATCTCCATGCAGTTGGACGATATCAATGCCGT
AATCATTGACCAGAGCCAAAACATCCTCCTTAGGTTGATTACGAAACACGCCAACCAAGT
ATTTCCGAGTGCCCTGAACATTTTTATATGCTTTTACAAGACTTGAAATTTTCTTGCAA
TAACCGGGTCAATTGTTCTCTTTCTATTGGGCACACATATAATACCCAGCAAGTCAGCAT
CGGAATCTAGAGCACATTCTGCGGCCCTCTGTGCTCTGCAAGCCGCAAACCTTTCACCAATG
GACCAGAACTACCTGTGAATTAATAACAGACATACTCCAAGCTGCCTTTGTGTGCTTAA
TCACGTATACTCACGTGCTCAATAGTCACCAATGCCCTCCCTCTTGGCCCTCTCCTTTTC
TTTTTTTCGACCGAATTAATTCTTAATCGGCAAAAAAAGAAAGCTCCGGATCAAGATTGT
ACGTAAGGTGACAAGCTATTTTTCAATAAAGAATATCTTCCACTACTGCCATCTGGCGTC
ATAACTGCAAAGTACACATATATTACGATGCTGTCTATTAAATGCTTCCTATATTATATA
TATAGTAATGTCGTTTATGGTGCACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAA
GCCAGCCCCGACACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGG
CATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTGAGAGGTTTTCAC
CGTCATCACCGAAACGCGCGAGACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTA
ATGTCATGATAATAATGGTTTCTTAGGACGGATCGCTTGCCTGTAACCTACACGCGCCTC
GTATCTTTTAAATGATGGAATAATTTGGGAATTTACTCTGTGTTTATTTATTTTATGTTT
TGTATTTGGATTTTAGAAAGTAAATAAAGAAGGTAGAAGAGTTACGGAATGAAGAAAAA
AAATAAACAAAGGTTTAAAAAATTTCAACAAAAGCGTACTTTACATATATATTTATTAG
ACAAGAAAAGCAGATTAAATAGATATACATTCGATTAAAGATAAGTAAATGTAAATCA
CAGGATTTTCGTGTGTGGTCTTCTACACAGACAAGATGAAACAATTCGGCATTAATACCT
GAGAGCAGGAAGAGCAAGATAAAAGGTAGTATTTGTTGGCGATCCCCCTAGAGTCTTTTA
CATCTTCGAAAAACAAAACTATTTTTCTTAATTTCTTTTTTTACTTTCTATTTTTAA
TTTATCTTTTATATGATAAATAATTTAAATTATAATTATTTTATAGCACGTGATGAAAAG
GACCCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTCTAA
ATACATTCAAATATGTATCCGCTCATGAGACAATAACCCGTATAAATGCTTCAATAATAT
TGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCG
GCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAA
GATCAGTTGGGTGCACGAGTGGGTACATCGAAGTGGATCTCAACAGCGGTAAGATCCTT
GAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGT
GGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTGCGCGCATACACTAT
TCTCAGAAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATG
ACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTA
CTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTTTCAACATGGGGGAT
CATGTAACCTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAG
CGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACCTATTAACCTGGCGAA
CTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCA
GGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCC
GGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGT
ATCGTAGTTATCTACACGACGGGCAGTCAGGCAACTATGGATGAACGAAATAGACAGATC
GCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACCTGTCAGACCAAGTTTACTCATAT
ATACTTTAGATTGATTTAAAACTTCATTTTAAATTTAAAGGATCTAGGTGAAGATCCTT
TTTGATAATCTCATGACCAAATCCCTTAACGTGAGTTTTTCGTTCCACTGAGCGTCAGAC
CCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGC
TTGCAAAACAAAAAACCACCGCTACCAGCGGTGGTTTGTGTTGCCGGATCAAGAGCTACCA
ACTCTTTTTCCGAAGGTAACCTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCTCTCTA
GTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCT
CTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTG
GACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTGGGCTGAACGGGGGGTTTCGTGC
ACACAGCCCAGCTTGGAGCGAACGACCTACACCGAAGTGAATACCTACAGCGTGAGCAT-

Figure 95B

TGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGG
GTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGGAACGCCTGGTATCTTTATAGT
CCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTGTGATGCTCGTCAGGGGGG
CCGAGCCTATGGA AAAACGCCAGCAACGCGGCCCTTTTACGGTTCTTGCCCTTTTGCTGG
CCTTTTGTCTACATGTTCTTCTGCGTTATCCCTGATTCTGTGGATAACCGTATTACC
GCCTTTGAGTGAGCTGATACCGCTCGCCGAGCCGAACGACCGAGCGCAGCGAGTCAGTG
AGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATT
CATTAATGCAGCTGGCAGCACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCA
ATTAATGTGAGTTACCTCACTCATTAGGCACCCAGGCTTTACACTTTATGCTTCCGGCT
CCTATGTTGTGTGGAATTGTGAGCGGATAACAATTTACACAGGAAACAGCTATGACCAT
GATTACGCCAAGCTCGGAATTAACCTCACTAAAGGGAACAAAAGCTGGGTACCGGGCCC
CCCCTCGAGATCCGGGATCGAAGAAATGATGGTAAATGAAATAGGAAATCAAGGAGCATG
AAGGCAAAAAGCAAATATAAGGGTCGAACGAAAAATAAAGTGAAAAGTGTTGATATGATG
TATTTGGCTTTGCGGCGCCGAAAAACAGAGTTTACGCAATTGCACAATCATGCTGACTCT
GTGGCGGACCCGCGCTCTTGCCGCGCCGCGGATAACGCTGGGCGTGAGGCTGTGCCCGGC
GGAGTTTTTTGCGCCTGCATTTTCCAAGGTTTACCTGCGCTAAGGGGCGAGATTGGAGA
AGCAATAAGAATGCCGGTTGGGGTTGCGATGATGACGACCACGACAACCTGGTGTCAATT
TTAAGTTGCCGAAAGAACCTGAGTGCAATTGCAACATGAGTATACTAGAAGAATGAGCCA
AGACTGCGGAGACCGGAGTTTGCCGGTGGTGCGAACAATAGAGCGACCATGACCTTGAAG
GTGAGACGCGCATAACCGCTAGAGTACTTTGAAGAGGAAACAGCAATAGGGTTGCTACCA
GTATAAATAGACAGGTACATACAACACTGGAAATGGTTGTCTGTTTGTGAGTACGCTTTCAA
TTCATTTGGGTGTGCACTTTATTATGTTACAATATGGAAGGGAACCTTTACACTTCTCCTA
TGCACATATATTAATTAAAGTCCAATGCTAGTAGAGAAGGGGGGTAACACCCCTCCGCGC
TCTTTTCCGATTTTTTTCTAAACCGTGGAATATTTCCGATATCCTTTTGTGTTTCCGGG
TGTAACAATATGGACTTCTCTTTCTGGCAACCAAACCCATACATCGGGATTCTCATAAT
ACCTTCGTTGGTCTCCCTAACATGTAGGTGGCGGAGGGGAGATATAACAATAGAACAGATA
CCAGACAAGACATAATGGGCTAAACAAGACTACACCAATTACACTGCCTCATTGATGGTG
GTACATAACGAACTAATACTGTAGCCCTAGACTTGATAGCCATCATCATATCGAAGTTTC
ACTACCCCTTTTTCCATTTGCCATCTATTGAAGTAATAATAGGCGCATGCAACTTCTTTTC
TTTTTTTTTCTTTCTCTCTCCCCGTTGTTGTCTCACCATATCCGCAATGACAAAAAA
ATGATGGAAGACACTAAAGGAAAAAATTAACGACAAAGACAGCACCAACAGATGTCGTTG
TTCCAGAGCTGATGAGGGGTATCTTCGAACACACGAAACTTTTCTTCTCCTTCAATCAG
CACACTACTCTAATGAGCAACGGTATACGGCCTTCTTCCAGTTACTTGAATTTGAAA
TAAAAAAGTTTGCCGCTTTGCTATCAAGTATAAATAGACCTGCAATTATTAATCTTTTG
TTTCTCGTCATTTGCTCTGTTCCCTTTCTTCTTCTGTTTCTTTTCTGCACAATATTTCA
AGCTATACCAAGCATACAATCAACTCCAAGCTTATGCCAAGAAGAAGCGGAAGGTCTCG
AGCGGCGCCAATTTTAATCAAAGTGGAATATTGCTGATAGCTCATTGTCCTTCACTTTC
ACTAACAGTAGCAACGGTCCGAACCTCATAACAACCTCAACAAATTTCTAAGCGCTTTC
CAACCAATTGCTCTCTCTAACGTTTCTGATAACTTCAATGAATAATGAAATCACGGCTAGT
AAAATTGATGATGGTAATAATTCAAACCACTGTACCTGGTTGGACGGACCAAACTGCG
TATAACGCGTTTGAATCACTACAGGGATGTTTAAATACCACTACAATGGATGATGTATAT
AACTATCTATTGATGATGAAGATACCCCAACCAACCAAAAAAGAGGGTGGGTGCAAT
CAACAAGTTTGTACAAAAAGCTGAACGAGAAACGTAAAATGATATAAATATCAATATA
TTAAATTAGATTTTGATAAAAAACAGACTACATAAATCTGTAAACACAACATATCCAG
TCACTATGGCGGCCGCTAAGTTGGCAGCATCACCCGACGCACTTTGCGCCGAATAAATAC
CTGTGACGGAAGATCACTTCGCAGAATAAATAAATCCTGGTGTCCCTGTTGATACCGGGA
AGCCCTGGGCCAACTTTTGGCGAAAATGAGACGTTGATCGGCACGTAAGAGGTTCCAAC
TTCACCATAAATGAAATAAGATCACTACCGGGCGTATTTTTTGTGATTATCGAGATTTTCAG
GAGCTAAGGAAGCTAAATGGAGAAAAAATCACTGGATATAACCACCGTTGATATATCCC
AATGGCATCGTAAAGAACATTTTGGAGGCATTTTCAGTCAGTTGCTCAATGTACCTATAACC
AGACCGTTTCAGCTGGATATTACGGCCTTTTTTAAAGACCGTAAAGAAAAATAAGCACAGT
TTTATCCGGCCTTTATTACATTCTTGCCCGCTGATGAATGCTCATCCGGAATTCGGTA
TGGCAATGAAAGACGGTGAGCTGGTGATATGGGATAGTGTTACCCCTGTTACACCGTTT
TCCATGAGCAAACTGAAACGTTTTTCATCGCTCTGGAGTGAATACCACGACGATTTCCGGC
AGTTTCTACACATATATTGCAAGATGTGGCGTGTACGGTGAAAACCTGGCCTATTTC
CTAAAGGGTTTATTGAGAATATGTTTTCTGCTCAGCCAATCCCTGGGTGAGTTTACCA
GTTTTGATTTAAACGTGGCCAATATGGACAACCTTCTCGCCCCCGTTTTTACCATGGGCA
AATATTATACGCAAGGCGACAAGGTGCTGATGCCGCTGGCGATTACAGGTTTCATCATGCCG-

FIGURE 95C

TCTGTGATGGCTTCCATGTCCGGCAGAATGCTTAATGAATTACAACAGTACTGCGATGAGT
GGCAGGGCGGGGCGTAATCTAGAGGATCCGGCTTACTAAAAGCCAGATAACAGTATGCGT
ATTTGCGCGCTGATTTTTGCGGTATAAGAATATATACTGATATGTATACCCGAAGTATGT
CAAAAAGAGGTGTGCTATGAAGCAGCGTATTACAGTGACAGTTGACAGCGACAGCTATCA
GTTGCTCAAGGCATATATGATGTCAATATCTCCGGTCTGGTAAGCACAACCATGCAGAAT
GAAGCCCGTCTGCTGCGTGCCGAACGCTGGAAAGCGGAAAATCAGGAAGGGATGGCTGAG
GTCGCCCCGTTTTATTGAAATGAACGGCTCTTTTGCTGACGAGAACAGGGACTGGTGAAAT
GCAGTTTAAGGTTTACACCTATAAAAGAGAGAGCCGTTATCGTCTGTTTGTGGATGTACA
GAGTGATATTATTGACACGCCCCGGGCGACGGATGGTGATCCCCCTGGCCAGTGACAGTCT
GCTGTCCAGATAAAGTCTCCCGTGAACCTTTACCCGGTGGTGCATATCGGGGATGAAAGCTG
GCGCATGATGACCACCGATATGGCCAGTGTGCCGGTCTCCGTTATCGGGGAAGAAGTGGC
TGATCTCAGCCACCGCGAAAATGACATCAAAAACGCCATTAACCTGATGTTCTGGGGAAT
ATAAATGTCAGGCTCCGTTATACACAGCCAGTCTGCAGGTGACCATAGTGACTGGATAT
GTTGTGTTTTACAGTATTATGTAGTCTGTTTTTTATGCAAAATCTAATTTAATATATTGA
TATTTATATCATTTTACGTTTCTCGTTTCTGTTTCTTGTACAAAGTGGTTTGTATGGCCGC
TAAGTAAGTAAGACGTCGAGCTCCCTATAGTGAGTCGTATTACACTGGCCGTCGTTTTTAC
AACGTCGTGACTGGGAAAACACCGGTGAGCTCTAAGTAAGTAACGGCCGCCACCGCGGTG
GAGCTTTGGACTTCTTCGCCAGAGGTTTGGTCAAGTCTCCAATCAAGGTTGTCCGCTTGT
CTACCTTGCCAGAAATTTACGAAAAGATGGAAAAGGGTCAAATCGTTGGTAGATACGTTG
TTGACACTTCTAAATAAGCGAATTTCTTATGATTTTATGATTTTTATTATTAAATAAGTTA
TAAAAAAATAAGTGTATACAAATTTTAAAGTGAAGTCTTAGGTTTTTAAACGAAAATTCT
TGTTCTTGAGTAACTCTTTCCTGTAGGTGAGGTTGCTTTCTCAGGTATAGCATGAGGTCG
CTCTTATTGACCACACCTCTACCGGCATGCCGAGCAAATGCCTGCAAATCGCTCCCCATT
TCACCCAATTGTAGATATGCTAACTCCAGCAATGAGTTGATGAATCTCGGTGTGTATTTT
ATGTCCTCAGAGGACAATACCTGTTGTAATCGTTCTTCCACACGGATCCGCATCAGGCGA
AATTGTAAACGTTAATATTTTGTAAATTCGCGTTAAATATTTGTTAAATCAGCTCATT
TTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAATCAAAGAATAGACCGAGAT
AGGGTTGAGTGTGTTCCAGTTTGGAAACAAGAGTCCACTATTAAAGAACGTGGACTCCAA
CGTCAAAGGGCGAAAAACCGTCTATCAGGGCGATGGCCCACTACGTGAACCATCACCTA
ATCAAGTTTTTTGGGGTCCAGGTGCCGTAAAGCACTAAATCGGAACCCTAAAGGGAGCCC
CCGATTTAGAGCTTGACGGGGAAAGCCGGCGAACGTGGCGAGAAAGGAAGGGAAGAAAGC
GAAAGGAGCGGCGCTAGGGCGCTGGCAAGTGTAGCGGTACAGCTGCGCGTAACCACCAC
ACCCGCCGCGCTTAATGCGCCGCTACAGGGCGCGTCCCATTCGCCATTCACTGCA

FIGURE 95D

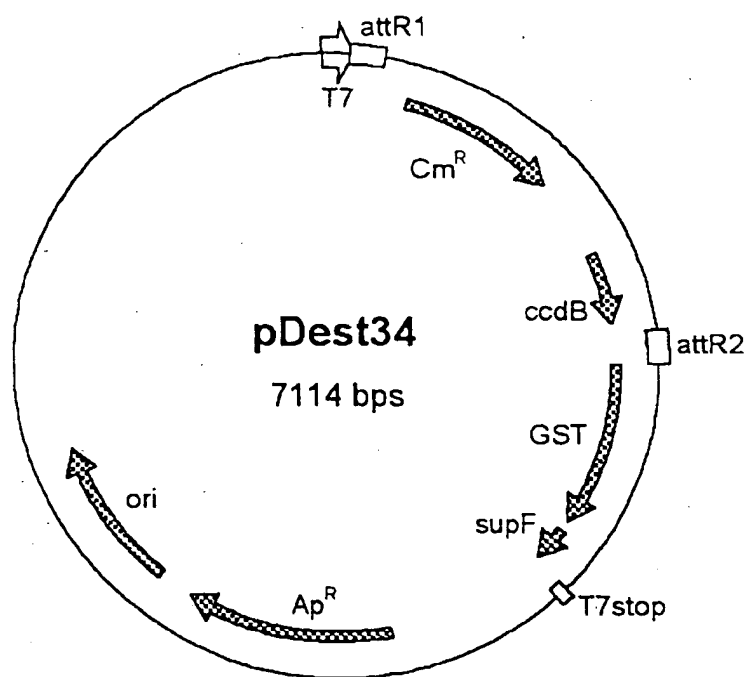


FIGURE 96A

pDEST34 7114 bp

<u>Location (Base Nos.)</u>	<u>Gene Encoded</u>
195..71	attR1
304..963	CmR
1305..1610	ccdB
1651..1775	attR2
1780..2472	GST
2675..2720	T7stop
3334..4194	ampR
4343..4982	ori

ATCGAGATCTCGATCCCGCGAAATTAATACGACTCACTATAGGGAGACCACAACGGTTTC
 CCTCTAGATCACAAAGTTTGTACAAAAAGCTGAACGAGAAACGTAAATGATATAAATAT
 CAATATATTAAATTAGATTTTGCATAAAAAACAGACTACATAATACTGTAAACACAACA
 TATCCAGTCACTATGGCGGCCGATTAGGCACCCAGGCTTTACACTTTATGCTTCCGGC
 TCGTATAATGTGTGGATTTTGTAGTTAGGATCCGCGAGATTTTCAGGAGCTAAGGAAGCT
 AAAATGGAGAAAAAATCACTGGATATACCACCGTTGATATATCCCAATGGCATCGTAAA
 GAACATTTTGTAGGCATTTTCACTCAGTTGCTCAATGTACCTATAACCAGACCGTTTCAGCTG
 GATATTACGGCCTTTTAAAGACCGTAAAGAAAAATAAGCACAAGTTTATCCGGCCTTTT
 ATTCACATTCTTGCCCGCTGATGAATGCTCATCCGGAATTCCGTATGGCAATGAAAGAC
 GGTGAGCTGGTGATATGGGATAGTGTTCACCCCTGTTACACCGTTTTCCATGAGCAAAC
 GAAACGTTTTTCATCGCTCTGGAGTGAATACCACGACGATTTCCGGCAGTTTCTACACATA
 TATTCGCAAGATGTGGCGTGTACGGTGAAAAACCTGGCCTATTTCCCTAAAGGGTTTATT
 GAGAATATGTTTTTTCGTCTCAGCCAATCCCTGGGTGAGTTTACCAGTTTTGATTTAAAC
 GTGGCCAATATGGACAACCTTCTCGCCCCCGTTTTTACCATGGGCAAATATTATACGCAA
 GCGGACAAGGTGCTGATGCCGCTGGCGATTTCAGGTTTCATCATGCCGTCTGTGATGGCTTC
 CATGTCCGCAGAATGCTTAATGAATTACAACAGTACTGCGATGAGTGGCAGGGCGGGGCG
 TAAACGCGTGATCCGGCTTACTAAAAGCCAGATAACAGTATGCGTATTTGCGCGCTGAT
 TTTTGGCGTATAAGAATATATACTGATATGTATACCCGAAGTATGTCAAAAAGAGGTGTG
 CTATGAAGCAGCGTATTACAGTGACAGTTGACAGCGACAGCTATCAGTTGCTCAAGGCAT
 ATATGATGTCAATATCTCCGGTCTGGTAAGCACCAACATGCAGAATGAAGCCCGTCTGCT
 GCGTGCCGAACGCTGGAAAGCGGAAAAATCAGGAAGGGATGGCTGAGGTGCGCCCGGTTTAT
 TGAAATGAACGGCTCTTTTGTGACGAGAACAGGGACTGGTGAAATGCAGTTTAAAGTTT
 ACACCTATAAAAAGAGAGAGCCGTTATCGTCTGTTTGTGGATGTACAGAGTGATATTATG
 ACACGCCCCGGGCGACGGATGGTGATCCCCCTGGCCAGTGACAGTCTGCTGTGATATAAG
 TCTCCCGTGAACCTTACCCGGTGGTGATATCGGGGATGAAAGCTGGCGCATGATGACCA
 CCGATATGGCCAGTGTGCCGCTCTCCGTTATCGGGGAAGAAGTGGCTGATCTCAGCCACC
 GCGAAAAATGACATCAAAAACGCCATTAAACCTGATGTTCTGGGGAATATAAATGTCAGGCT
 CCCTTATACACAGCCAGTCTGCGAGTTCGACCATAGTGACTGGATATGTTGTGTTTTACAG
 TATTATGTAGTCTGTTTTTTATGCAAAATCTAATTTAATATATTGATATTTATATCATTT
 TACGTTTCTCGTTTCACTTCTTGTACAAAGTGGTGATTATGTCCCCTATACTAGGTTAT
 TGGAAAATTAAGGGCCTTGTGCAACCCACTCGACTTCTTTTGAATATCTTGAAGAAAAA
 TATGAAGAGCATTTGTATGAGCGCGATGAAGGTGATAAATGGCGAAACAAAAAGTTTGAA
 TTGGGTTTGGAGTTTCCCAATCTTCTTATTATATTGATGGTGATGTTAAATTAACACAG
 TCTATGGCCATCATACGTTATATAGCTGACAAGCACACATGTTGGGTGGTTGTCCAAAA
 GAGCGTGCAGAGATTTCAATGCTTGAAGGAGCGGTTTTGGATATTAGATACGGTGTTTCG
 AGAATTGCATATAGTAAAGACTTTGAAACTCTCAAAGTTGATTTTCTTAGCAAGCTACCT
 GAAATGCTGAAAATGTTTCAAGATCGTTTATGTCATAAAACATATTTAAATGGTGATCAT
 GTAACCCATCTGACTTCATGTTGTATGACGCTCTTGATGTTGTTTTATACATGGACCCA
 ATGTGCCTGGATGCGTTCCCAAAATTAGTTTGTTTTAAAAAACGTATTGAAGCTATCCCA
 CAAATTGATAAGTACTTGAATCCAGCAAGTATATAGCATGGCCTTTGCAGGGCTGGCAA
 GCCAGCTTTGGTGGTGCGACCATCTCCAAAAATCGGATCTGGTTCCGCGTCCATGGGGA
 TCCGCGTGCAGAAAGCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCGCTT
 CCCGATAAGGGAGCAGGCCAGTAAAGCATTACCCGTGGTGGGGTTCCCGAGCGGCCAAA
 GGGAGCAGACTCTAAATCTGCCGTATCGACTTCGAAGGTTTGAATCCTTCCCCACCAC
 CATCACTTTCAAAGTGAATTCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAA-

FIGURE 96B

ACGGGTCTTGAGGGGTTTTTTTGCTGAAAGGAGGAACATATCCGGATATCCACAGGACGG
GTGTGGTCCCATGATCGCGTAGTCGATAGTGGCTCCAAGTAGCGAAGCGAGCAGGACTG
GGCGGCGCCAAAGCGGTCCGACAGTGTCTCCGAGAACGGGTGCGCATAGAAATTGCATCA
ACGCATATAGCGCTAGCAGCACGCCATAGTGAAGTGGCGATGCTGTGCGAATGGACGATAT
CCCGCAAGAGGCCCGGCAGTACCGGCATAACCAAGCCTATGCCTACAGCATCCAGGGTGA
CGGTGCCGAGGATGACGATGAGCGCATTGTTAGATTTATACACGGTGCCTGACTGCGTT
AGCAATTTAACTGTGATAAACTACCGCATTAAAGCTTATCGATGATAAGCTGTCAAACAT
GAGAATTCTTGAAGACGAAAGGGCCTCGTGATACGCCTATTTTATAGGTTAATGTCATG
ATAATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCT
ATTTGTTTATTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCCTGA
TAAATGCTTCAATAATATTGAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTGCGC
CTTATTCCCTTTTTTGCGGCATTTTGCTTCTGTTTTTGGCTCACCAGAAACGCTGGTG
AAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTACATCGAACTGGATCTC
AACAGCGGTAAAGATCCTTGAGAGTTTTCGCCCCGAAGAAGCTTTTCCAATGATGAGCACT
TTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTGTGACGCCGGGCAAGAGCAACTC
GGTCCGCCCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAG
CATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGAT
AACATGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTT
TTGCACAACATGGGGGATCATGTAACCTCGCCTTGATCGTTGGGAACCGGAGCTGAAGAA
GCCATACCAAACGACGAGCGTGACACCAGATGCCTGCAGCAATGGCAACAACGTTGCGC
AAACTATTAAGTGGCGAACTACTTACTCTAGCTTCCCGCAACAATTAATAGACTGGATG
GAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATT
GCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATGTCAGCACTGGGGCCA
GATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGAT
GAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCA
GACCAAGTTTACTCATATATACTTTAGATTGATTTAAACTTCATTTTTTAATTTAAAGG
ATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCG
TTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTT
CTGCGCGTAATCTGCTGCTTGCAAACAAAAAACACCGCTACCAGCGGTGGTTTGTGTTG
CCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAAGTGGCTTACGAGAGCGCAGATA
CCAAATACTGTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCA
CCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAG
TCGTGTCTTACCGGGTTGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGCTCGGGC
TGAACGCGGGGGTTCTGTGCACACAGCCAGCTTGGAGCGAACGACCTACACCGAACTGAGA
TACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGG
TATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAAC
GCCTGGTATCTTTATAGTCTGTGCGGTTTTCGCCACCTCTGACTTGAGCGTCGATTTTTG
TGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGG
TTCTGCGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTCTGCGTTATCCCTGATTCT
GTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGAGCCGAACGACC
GAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCTGATGCGGTATTTTCTCCTT
ACGCATCTGTGCGGTATTTACACCGCATATATGGTGCACCTCTCAGTACAATCTGCTCTG
ATGCCGCATAGTTAAGCCAGTATACACTCCGCTATCGCTACGTGACTGGGTCTGGCTGC
GCCCCGACACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATC
CGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTGAGAGGTTTTACCGTC
ATCACCGAAACGCGCGAGGCAGCTGCGGTAAAGCTCATCAGCGTGGTCTGTAAGCGATT
ACAGATGTCTGCCTGTTTATCCGCGTCCAGCTCGTTGAGTTTCTCCAGAAGCGTTAATGT
CTGGCTTCTGATAAAGCGGGCCATGTTAAGGGCGTTTTTTTCTGTTTGGTCACTGATGC
CTCCGTGTAAGGGGGATTTCTGTTTATGGGGTAATGATACCGATGAAACGAGAGAGGAT
GCTCACGATACGGGTACTGATGATGAACATGCCCGGTTACTGGAACGTTGTGAGGGTAA
ACAAGTGGCGGTATGGATGCGGCGGGACAGAGAAAAATCACTCAGGGTCAATGCCAGCG
CTTCGTTAATACAGATGTAGGTGTTCCACAGGGTAGCCAGCAGCATCCTGCGATGCAGAT
CCGGAACATAATGGTGCAGGGCGCTGACTTCCGCGTTTCCAGACTTTACGAAACACGGAA
ACCGAAGACCATTCATGTTGTTGCTCAGGTGCGAGACGTTTTGCGAGCAGCAGTCTGCTCA
CGTTCGCTCGCGTATCGGTGATTCTTCTGCTAACAGTAAGGCAACCCCGCCAGCCTAG
CCGGGTCTCAACGACAGGAGCAGCATGCGCACCCCGTGGCCAGGACCCCAACGCTGCC
CGAGATGCGCCGCTGCGGCTGCTGGAGATGGCGGACGCGATGGATATGTTCTGCCAAGG
GTTGGTTTGCGCATTACAGTTCTCCGCAAGAATTGATTGGCTCCAATTCTTGGAGTGGT-

FIGURE 96C

GAATCCGTTAGCGAGGTGCCGCCGGCTTCCATTACAGGTCGAGGTGGCCCGGCTCCATGCA
CCGCGACGCAACGCGGGGAGGCAGACAAGGTATAGGGCGGCGCTACAATCCATGCCAAC
CCGTTCCATGTGCTCGCCGAGGCGGCATAAATCGCCGTGACGATCAGCGGTCCAGTGATC
GAAGTTAGGCTGGTAAGAGCCGCGAGCGATCCTTGAAGCTGTCCCTGATGGTCGTCTCT
ACCTGCCTGGACAGCATGGCCTGCAACGCGGGCATCCCGATGCCGCCGGAAGCGAGAAGA
ATCATAATGGGGAAGGCCATCCAGCCTCGCGTCGCGAACGCCAGCAAGACGTAGCCCAGC
GCGTCGGCCCGCATGCCGGCGATAATGGCCTGCTTCTCGCCGAAACGTTTGGTGGCGGGA
CCAGTGACGAAGGCTTGAGCGAGGGCGTGCAAGATTCCGAATACCGCAAGCGACAGGCCG
ATCATCGTCGCGCTCCAGCGAAAGCGGTCTCGCCGAAAATGACCCAGAGCGCTGCCGGC
ACCTGTCTACGAGTTGCATGATAAAGAAGACAGTCATAAGTGCGGCGACGATAGTCATG
CCCCGCGCCACCGGAAGGAGCTGACTGGGTTGAAGGCTCTCAAGGGCATCGGTGATCG
ACGCTCTCCCTTATGCGACTCCTGCATTAGGAAGCAGCCCAGTAGTAGGTTGAGGCCGTT
GAGCACCGCCGCGCAAGGAATGGTGCATGCAAGGAGATGGCGCCCAACAGTCCCCCGGC
CACGGGGCCTGCCACCATACCACGCCGAAACAAGCGCTCATGAGCCCGAAGTGGCGAGC
CCGATCTTCCCCATCGGTGATGTGCGCGATATAGGCGCCAGCAACCGCACCTGTGGCGCC
GGTGATGCCGGCCACGATGCGTCCGGCGTAGAGG

FIGURE 96a

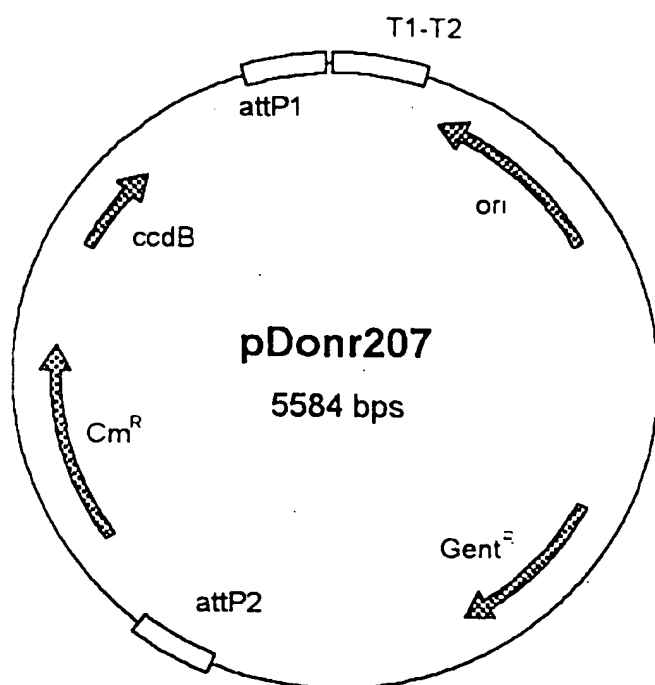


FIGURE 97A

pDONR207

5584 bp

GCGAGAGTAGGGAAGTCCAGGCATCAAATAAAACGAAAGGCTCAGTCGGAAGACTGGGC
CTTTCGTTTTATCTGTTGTTTGTGCGGTGAACGCTCTCCTGAGTAGGACAAATCCGCCGGG
AGCGGATTTGAACGTTGTGAAGCAACGGCCCGGAGGGTGGCGGGCAGGACGCCCGCCATA
AACTGCCAGGCATCAAATAAGCAGAAGGCCATCCTGACGGATGGCCTTTTTGCGTTTCT
ACAAACTCTTCTGGCTAGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGA
AAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTG
GCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAG
AGGTGGCGAAACCCGACAGGACTATAAGATACAGGCGTTTCCCCCTGGAAGCTCCCTC
GTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCG
GGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTT
CGCTCCAAGCTGGGCTGTGTGCACGAACCCCCGTTAGCCCCGACCGCTGCGCCTTATCC
GGTAACATATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCC
ACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGG
TGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCA
GTTACCTTCGGA AAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGC
GGTGGTTTTTTTGTGTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGAT
CCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGAACGAAAACCTCACGTTAAGGGATT
TTGGTCATGAGCTTGCGCCGTCCCGTCAAGTCAGCGTAATGCTCTGCCAGTGTTACAACC
AATTAACCAATTCTGATTAGAAAACTCATCGAGCATCAAATGAACTGCAATTTATTCA
TATCAGGATTATCAATACCATATTTTTGAAAAAGCCGTTTCTGTAATGAAGGAGAAAACT
CACCGAGGCAGTTCCATAGGATGGCAAGATCCTGGTATCGGTCTGCGATTCCGACTCGTC
CAACATCAATAACAACCTATTAGTAGCCAAACCTAGAACTATAGCTAGAGTCTTGGGCGA
ACAAACGATGCTCGCCTTCCAGAAAAACCGAGGATGCGAACCACTTCATCCGGGGTCAGCA
CCACCGGCAAGCGCCGCGAGCGCCGAGGTCTTCCGATCTCCTGAAGCCAGGGCAGATCCG
TGCACAGCACCTTGCCGTAGAAGAACAGCAAGGCCGCCAATGCCTGACGATGCGTGGAGA
CCGAAACCTTGCGCTCGTTCGCCAGCCAGGACAGAAATGCCTCGACTTCGCTGCTGCCCA
AGGTGCGCGGTGACGCACACCGTGGAAACGGATGAAGGCACGAACCCAGTTGACATAAG
CCTGTTCCGGTTCGTAAACTGTAATGCAAGTAGCGTATGCGCTCACGCAACTGGTCCAGAA
CCTTGACCGAACGCAGCGGTGGTAACGGCGCAGTGGCGGTTTTTCATGGCTTGTTATGACT
GTTTTTTGATGTACAGTCTATGCCCTCGGGCAGCAAGCGCGTTACGCCGTGGGTC
GATGTTTGATGTTATGGAGCAGCAACGATGTTACGCGCAGCAACGATGTTACGCAGCAG
GGCAGTCGCCCTAAAACAAAGTTAGGTGGCTCAAGTATGGGCATCATTGCGACATGTAGG
CTCGGCCCTGACCAAGTCAAATCCATGCGGGCTGCTCTTGATCTTTTCGGTCGTGAGTTC
GGAGACGTAGCCACCTACTCCCAACATCAGCCGACTCCGATTACCTCGGGAACTTGCTC
CGTAGTAAGACATTATCGCGCTTGCTGCCTTCGACCAAGAAGCGGTTGTTGGCGCTCTC
GCGGCTTACGTTCTGCCAGGTTTGAGCAGCCGCGTAGTGAGATCTATATCTATGATCTC
GCAGTCTCCGGCGAGCACCGGAGGCAGGGCATTGCCACCGCGCTCATCAATCTCCTCAAG
CATGAGGCCAACCGCTTGTTGCTTATGTGATCTACGTGCAAGCAGATTACGGTGACGAT
CCCGCAGTGCGTCTCTATACAAAGTTGGGCATACGGGAAGAAGTGATGCACCTTGATATC
GACCCAAGTACCGCCACCTAACAATTGTTCAAGCCGAGATCGGCTTCCCGGCCTAATTT
CCCCTCGTCAAAAATAAGGTTATCAAGTGAGAAATCACCATGAGTGACGACTGAATCCGG
TGAGAATGGCAAAAGTTTATGCATTTCTTCCAGACTTGTTCAACAGGCCAGCCATTACG
CTCGTCATCAAAATCACTCGCATCAACCAAACCGTTATTTCATTCTGATTGCGCCTGAGC
GAGACGAAATACGCGATCGCTGTTAAAGGACAATTACAAACAGGAATCGAATGCAACCG
GCGCAGGAACACTGCCAGCGCATCAACAATATTTTACCTGAATCAGGATATTCTTCTAA
TACCTGGAATGCTGTTTTTCCGGGGATCGCAGTGGTGAGTAACCATGCATCATCAGGAGT
ACGGATAAAATGCTTGATGGTGGGAAGAGGCATAAATTCCGTCAGCCAGTTTAGTCTGAC
CATCTCATCTGTAACATCATTGGCAACGCTACCTTTGCCATGTTTCAGAAACAACCTCTGG
CGCATCGGGCTTCCCATACAAGCGATAGATTGTGCGACCTGATTGCCCGACATTATCGCG
AGCCCATTTATACCCATATAAATCAGCATCCATGTTGGAATTTAATCGCGGCCTCGACGT
TTCCCGTTGAATATGGCTCATAACACCCCTGTATTACTGTTTATGTAAGCAGACAGTTT
TATTGTTTCATGATGATATATTTTATCTTGTCATGTAACATCAGAGATTTTGAGACAC
GGGCGTAGCTGAGCTGGATGGCAATAATGATTATTTTACTGATGATAGTGACCTGTT
CGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGCTG
AACGAGAAACGTA AATGATATAAATATCAATATATTAATTAGATTTTGATAAAAAAC
AGACTACATAATACTGTAAACACAACATATCCAGTCACTATGAATCAACTACTTAGATG-

FIGURE 97B

GTATTAGTGACCTGTAGTCGACTAAGTTGGCAGCATCACCCGACGCACTTTGCGCCGAAT
AAATACCTGTGACGGAAGATCACTTCGCAGAATAAATAAATCCTGGTGTCCCTGTTGATA
CCGGGAAGCCCTGGGCCAACTTTGGCGAAAATGAGACGTTGATCGGCACGTAAGAGGTTTC
CAACTTTCACCATAATGAAATAAGATCACTACCGGGCGTATTTTTTTGAGTTATCGAGATT
TTCAGGAGCTAAGGAAGCTAAAAATGGAGAAAAAATCACTGGATATACCACCGTTGATAT
ATCCCAATGGCATCGTAAAGAACATTTTGAGGCATTTTCAGTCAGTTGCTCAATGTACCTA
TAACCAGACCGTTTCAGCTGGATATTACGGCCTTTTTTAAAGACCGTAAAGAAAAATAAGCA
CAAGTTTTATCCGGCCTTTATTACATTCTTGCCCGCCTGATGAATGCTCATCCGGAATT
CCGTATGGCAATGAAAGACGGTGAGCTGGTGTATGGGATAGTGTTTACCCTTGTTACAC
CGTTTTCCATGAGCAAACTGAAACGTTTTTCATCGCTCTGGAGTGAATACCACGACGATTT
CCGGCAGTTTCTACACATATATTGCAAGATGTGGCGTGTTACGGTGAAAACCTGGCCTA
TTTCCCTAAAGGGTTTATTGAGAATATGTTTTTCGTCTCAGCCAATCCCTGGGTGAGTTT
CACCAGTTTTGATTTAAACGTGGCCAATATGGACAACCTTCTTCGCCCCCGTTTTTACCAT
GGGCAAATATTATACGCAAGGCGACAAGGTGCTGATGCCGCTGGCGATTTCAGGTTTCATCA
TGCCGTCTGTGATGGCTTCCATGTCCGCAGAATGCTTAATGAATTACAACAGTACTGCGA
TGAGTGGCAGGGCGGGGCGTAATCGCGTGGATCCGGCTTACTAAAAGCCAGATAACAGTA
TGCGTATTTGCGCGCTGATTTTTTGCGGTATAAGAATATATACTGATATGTATACCCGAAG
TATGTCAAAAAGAGGTGTGCTATGAAGCAGCGTATTACAGTGACAGTTGACAGCGACAGC
TATCAGTTGCTCAAGGCATATATGATGTCAATATCTCCGGTCTGGTAAGCACAACCATGC
AGAATGAAGCCCGTCTGCTGCGTGCCGAACGCTGGAAAGCGGAAAATCAGGAAGGGATGG
CTGAGGTGCGCCCGTTTTATTGAAATGAACGGCTCTTTTGCTGACGAGAACAGGGACTGGT
GAAATGCAGTTTAAGGTTTACACCTATAAAAAGAGAGAGCCGTTATCGTCTGTTGTGGAT
GTACAGAGTGATATTATTGACACGCCCGGGCGACGGATGGTGATCCCCCTGGCCAGTGCA
CGTCTGCTGTGAGATAAAGTCTCCCGTGAACCTTTACCCGGTGGTGATATCGGGGATGAA
AGCTGGCGCATGATGACCACCGATATGGCCAGTGTGCCGGTCTCCGTTATCGGGGAAGAA
GTGGCTGATCTCAGCCACCGCGAAAATGACATCAAAAACGCCATTAACTGATGTTCTGG
GGAATATAAATGTGAGGCTCCCTTATACACAGCCAGTCTGCAGGTGATACAGTAGAAAT
TACAGAACTTTATCACGTTTAGTAAGTATAGAGGCTGAAAATCCAGATGAAGCCGAACG
ACTTGTAAAGAGAAAAGTATAAGAGTTGTGAAATTGTTCTTGATGCAGATGATTTTCAGGA
CTATGACACTAGCGTATATGAATAGGTAGATGTTTTTATTTTGTACACAAAAAAGAGGC
TCGCACCTCTTTTTCTTATTTCTTTTTATGATTTAATACGGCATTGAGGACAATAGCGAG
TAGGCTGGATACGACGATTCCGTTTGAGAAGAACATTTGGAAGGCTGTCGGTCTGACTAAG
TTGGCAGCATCACCCGAAGAACATTTGGAAGGCTGTCCGTGACTACAGGTCACTAATAC
CATCTAAGTAGTTGATTCATAGTGACTGGATATGTTGTGTTTTACAGTATTATGTAGTCT
GTTTTTTATGCAAAATCTAATTTAATATATTGATATTTATATCATTTTACGTTTCTCGTT
CAGCTTTTTTGTACAAAGTTGGCATTATAAAAAAGCATTGCTCATCAATTTGTTGCAACG
AACAGGTCACTATCAGTCAAAATAAAATCATTATTTGGGGCCCGAGATCCATGCTAGCGT
TAAC

FIGURE 97C

pMAB85

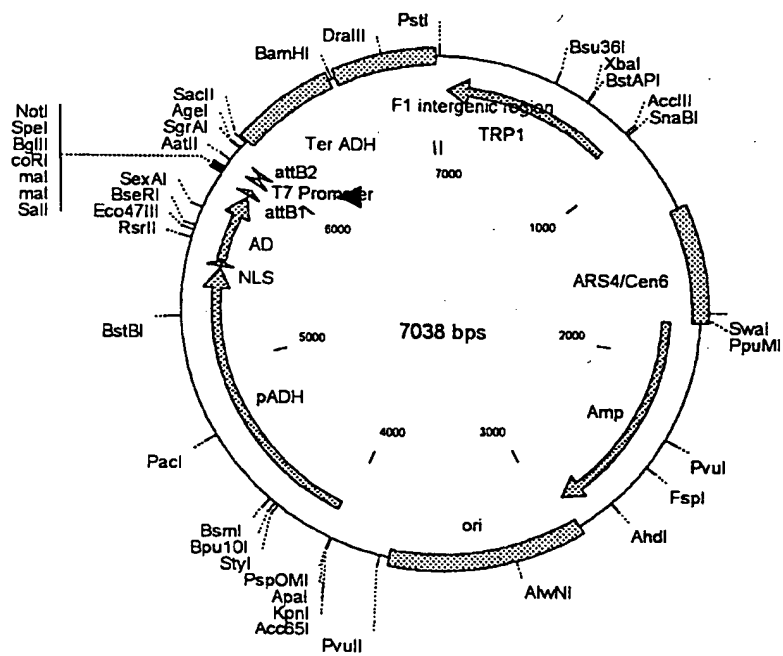


FIGURE 98A

pMAB85

7038 bp

GCCTTACGCATCTGTGCGGTATTTTCACACCGCAGGCAAGTGCACAAACAATACTTAAATA
AATACTACTCAGTAATAACCTATTTCTTAGCATTTTGTGACGAAATTTGCTATTTGTAG
AGTCTTTTACACCATTTGTCTCCACACCTCCGCTTACATCAACACCAATAACGCCATTTA
ATCTAAGCGCATCACCAACATTTTCTGGCGTCAGTCCACCAGCTAACATAAAATGTAAGC
TTTCGGGGCTCTCTTGCCCTTCCAACCCAGTCAGAAATCGAGTTCCAATCCAAAAGTTCAC
CTGTCCCACCTGCTTCTGAATCAAACAAGGGAATAAACGAATGAGGTTTCTGTGAAGCTG
CACTGAGTAGTATGTTGCACTCTTTTGGAAATACGAGTCTTTTAATAACTGGCAAACCGA
GGAACCTTGGTATTCTTGCCACGACTCATCTCCATGCAGTTGGACGATATCAATGCCGT
AATCATTGACCAGAGCCAAAACATCCTCCTTAGGTTGATTACGAAACACGCCAACCAAGT
ATTTTCGAGTGCCTGAACTATTTTTATATGCTTTTACAAGACTTGAAATTTTCTTGCAA
TAACCGGTCAATTGTTCTCTTTCTATTGGGCACACATATAATACCCAGCAAGTCAGCAT
CGGAATCTAGAGCACATTTCTGCGGCCTCTGTGCTCTGCAAGCCGCAAACCTTCCACCATG
GACCAGAATACTACGTGAAATTAATAACAGACATACTCCAAGCTGCCTTTGTGTGCTTAA
TCACGTATACTACGTGCTCAATAGTCACCAATGCCCTCCCTCTTGCCCTCTCCTTTTC
TTTTTTCGACCGAATTAATTCTTAATCGGCAAAAAAAGAAAGCTCCGGATCAAGATTGT
ACGTAAGGTGACAAGCTATTTTTCAATAAAGAATATCTTCCACTACTGCCATCTGGCGTC
ATAACTGCAAAGTACACATATATTACGATGCTGTCTATTAAATGCTTCCTATATTATATA
TATAGTAATGTGCTTTATGGTGCACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAA
GCCAGCCCCGACACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGG
CATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTGAGAGGTTTTAC
CGTCATCACCGAAACGCGGAGACGAAAGGCCCTCGTGATACGCCCTATTTTATAGGTTA
ATGTCATGATAAATAATGGTTTTCTTAGGACGGATCGCTTGCCTGTAACCTTACACGCGCCTC
GTATCTTTTAATGATGGAATAATTTGGGAATTTACTCTGTGTTTATTTATTTTATGTTT
TGTATTTGGATTTTAGAAAGTAAATAAAGAAGGTAGAAGAGTTACGGAATGAAGAAAAA
AAATAAACAAAGGTTTAAAAAATTTCAACAAAAGCGTACTTTACATATATATTTATTAG
ACAAGAAAAGCAGATTAAATAGATATACATTCGATTAAAGATAAGTAAATGTAAATCA
CAGGATTTTCGTGTGTGGTCTTCTACACAGACAAGATGAAACAATTCGGCATTAAATACCT
GAGAGCAGGAAGAGCAAGATAAAAGGTAGTATTTGTTGGCGATCCCCCTAGAGTCTTTTA
GAGCTTCGGAATAAATAAATACTATTTTCTTTAATTTCTTTTTTACTTCTATTTTTAA
TTTATATATTTATATTAAAAAATTTAAATTATAATTATTTTATAGCACGTGATGAAAAG
GACCCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAA
ATACATTCAAATATGTATCCGCTCATGAGACAATAACCCCTGATAAATGCTTCAATAATAT
TGAAAAGGAAGAGTATGAGTATTCACATTTCCGTGTGCGCCCTTATTCCTTTTTTGCG
GCATTTTGCTTCTGTTTTTGTCTACCCAGAAACGCTGGTGAAAGTAAAGATGCTGAA
GATCAGTTGGGTGCACGAGTGGGTTACACTGCAACTGGATCTCAACAGCGGTAAGATCCTT
GAGAGTTTTCGCCCCGAAGAACGTTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGT
GGCGCGGTATTATCCCGTATTGACGCGGGCAAGAGCAACTCGGTGCGCCGCATACACTAT
TCTCAGAATGACTTGGTTGAGTACTCACCAGTCAAGAAAAGCATCTTACGGATGGCATG
ACAGTAAGAGAATTATGCAGTGTGCCATAACCATGAGTGATAACACTGCGGCCAACTTA
CTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTTCACAACATGGGGGAT
CATGTAACCTGCGCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAG
CGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAACCTGGCGAA
CTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCA
GGACCACTTCTGCGCTCGGCCCTTCCGGCTGGTGGTTTATGTGCTGATAAATCTGGAGCC
GGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGT
ATCGTAGTTATCTACACGACGGGCAGTCAGGCAACTATGGATGAACGAAATAGACAGATC
GCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAAGTGTGAGACCAAGTTTACTCATAT
ATACTTTAGATTGATTTAAACTTCATTTTTTAATTTAAAGGATCTAGGTGAAGATCCTT
TTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGAC
CCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTCTGCGCGTAATCTGCTGC
TTGCAAAACAAAAACCAACCGCTACCAGCGGTGGTTTGTGTTGCGGATCAAGAGCTACCA
ACTCTTTTCCGAAGGTAAGTGGCTTTCAGCAGAGCGCAGATACCAAATACTGTCTTCTA
GTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCAGCTACATACCTCGCT
CTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTG
GACTCAAGACGATAGTTACCGGATAAGGCGCAGCGTCCGGCTGAACGGGGGTTCTGTC-

Figure 98B

ACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCAT
TGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGG
GTCCGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGGAACGCCTGGTATCTTTATAGT
CCTGTCCGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTGTGATGCTCGTCAGGGGGG
CCGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTACGGTTCCTGGCCTTTTGCTGG
CCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACC
GCCTTTGAGTGAGCTGATACCGCTCGCCGAGCCGAACGACCGAGCGCAGCGAGTCAGTG
AGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATT
CATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCA
ATTAATGTGAGTTACCTCACTCATTAGGCACCCAGGCTTTACACTTTATGCTTCCGGCT
CCTATGTTGTGTGGAATTGTGAGCGGATAACAATTTACACAGGAAACAGCTATGACCAT
GATTACGCCAAGCTCGGAATTAACCCCTCACTAAAGGGAACAAAAGCTGGGTACCGGGCCC
CCCCTCGAGATCCGGGATCGAAGAAATGATGGTAAATGAAATAGGAAATCAAGGAGCATG
AAGGCAAAAGACAAATATAAGGGTCAACGAAAAATAAAGTGAAAAGTGTTGATATGATG
TATTTGGCTTTGCGGCGCCGAAAAACGAGTTTACGCAATTGCACAATCATGCTGACTCT
GTGGCGGACCCGCGCTCTTGCCGGCCCGCGGATAACGCTGGGCGTGAGGCTGTGCCCGGC
GGAGTTTTTTGCGCCTGCATTTTCCAAGGTTTACCCTGCGCTAAGGGGCGAGATTGGAGA
AGCAATAAGAATGCCGGTTGGGGTTGCGATGATGACGACCACGACAACTGGTGTCAATTAT
TTAAGTTGCCGAAAGAACCTGAGTGCATTTGCAACATGAGTATACTAGAAGAATGAGCCA
AGACTTGCGAGACGCGAGTTTGCCGGTGGTGCGAACAATAGAGCGACCATGACCTTGAAG
GTGAGACGCGCATAACCGCTAGAGTACTTTGAAGAGGAAACAGCAATAGGGTTGCTACCA
GTATAAATAGACAGGTACATACAACACTGGAAATGGTTGTCTGTTTGAGTACGTTTTCAA
TTCATTTGGGTGTGCACTTTATTATGTTACAATATGGAAGGGAACCTTTACACTTCTCCTA
TGCACATATATTAATTAAGTCCAATGCTAGTAGAGAAGGGGGGTAACACCCCTCCGCGC
TCTTTTCCGATTTTTTTCTAAACCGTGGAATATTTCCGATATCCTTTTGTGTTTCCGGG
TGTAACATATGGACTTCCTCTTTTCTGGCAACCAAACCCATACATCGGGATTCTTATAAT
ACCTTCGTTGGTCTCCCTAACATGTAGGTGGCGGAGAGATATACAATAGAACAGATA
CCAGACAAGACATAATGGGCTAAACAAGACTACACCAATTACACTGCCTCATTGATGGTG
GTACATAACGAACTAATACTGTAGCCCTAGACTTGATAGCCATCATCATATCGAAGTTTC
ACTACCTTTTTTCCATTTGCCATCTATTGAAGTAATAATAGGCGCATGCAACTTCTTTTC
TTTTTTTTTCTTTTCTCTCTCCCCGTGTGTCTCACCATATCCGCAATGACAAAAAA
ATGATGGAAGACACTAAAGGAAAAAATTAACGACAAAGACAGCACCAACAGATGTCGTTG
TTCCAGAGCTGATGAGGGGTATCTTCGAACACACGAAACTTTTCTTCTCCTTCACTCAG
CACACTACTCTAATGAGCAACGGTATACGGCCTTCTTCCAGTTACTTGAATTTGAAA
TAAAAAAGTTTGGCGCTTTGCTATCAAGTATAAATAGACCTGCAATTATTAATCTTTTG
TTTCTCGTCATTGTTCTCGTTCCCTTTCTTCTTGTCTTTTCTGCACAATATTTCA
AGCTATACCAAGCATAAATCAACTCCAAGCTTATGCCCAAGAAGAAGCGGAAGGTCTCG
AGCGGCGCCAATTTTAATCAAAGTGGGAATATTGCTGATAGCTCATTGTCCTTCACTTTC
ACTAACAGTAGCAACGGTCCGAACCTCATAACAACCTCAACAAATTCTCAAGCGCTTTCA
CAACCAATTGCCCTCTAACGTTTCATGATAACTTCATGAATAATGAAATCACGGCTAGT
AAAATTGATGATGGTAATAATTCAAAACCACTGTACCTGGTTGGACGGACCAAACTGCG
TATAACGCGTTTGGAAATCACTACAGGGATGTTTAATACCACTACAATGGATGATGTATAT
AACTATCTATTTCGATGATGAAGATACCCCAACCAACCAAAAAAGAGGGTGGGTGCGATC
ACAAGTTTGTACAAAAAGCAGGCTTGTGACCCCGGAATTTCAGATCTACTAGTGCGGC
CGCACGCGTACCCAGCTTTCTGTACAAAGTGGTGACGTGAGCTCCCTATAGTGAGTCG
TATTACACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACACCGGTGAGCTCTAAGT
AAGTAACGGCCGCCACCGCGGTGGAGCTTTGGACTTCTTCGCCAGAGGTTTGGTCAAGTC
TCCAATCAAGGTTGTGCGCTTGTCTACCTTGCCAGAAATTTACGAAAAGATGGAAAAGGG
TCAAATCGTTGGTAGATACGTTGTTGACACTTCTAAATAAGCGAATTTCTTATGATTTAT
GATTTTTTATTATTAATAAGTTATAAAAAAATAAGTGTATACAAATTTTAAAGTGACTC
TTAGGTTTTTAAACGAAAATTTCTTGTCTTGAGTAACCTTTTCTGTAGGTCAGGTTGCT
TTCTCAGGTATAGCATGAGGTGCTCTTATTGACCACACCTCTACCGGCATGCCGAGCAA
ATGCCTGCAAATCGCTCCCATTTTCAACCAATTGTAGATATGCTAACTCCAGCAATGAGT
TGATGAATCTCGGTGTGTATTTTATGTCCTCAGAGGACAATACCTGTTGTAATCGTTCTT
CCACACGGATCCGCATCAGGCGAAATTGTAAACGTTAATATTTTGTAAATTCGCGTTA
AATATTTGTAAATCAGCTCATTTTTTAAACCAATAGGCCGAAATCGGCAAAATCCCTTAT
AAATCAAAGAATAGACCGAGATAGGGTTAGTGTGTTCCAGTTTGGAAACAGAGTCCA
CTATTAAGAACGTGGACTCCAACGTCAAAGGCGAAAAACCGTCTATCAGGGCGATGGC-

CCACTACGTGAACCATCACCCCTAATCAAGTTTTTTGGGGTCGAGGTGCCGTAAAGCACTA
AATCGGAACCCCTAAAGGGAGCCCCGATTTAGAGCTTGACGGGGAAAGCCGGCGAACGTG
GCGAGAAAGGAAGGGAAGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCG
GTCACGCTGCGCGTAACCACCACACCCGCCGCGCTTAATGCGCCGCTACAGGGCGCGTCC
CATTGCCATTCACTGCA

FIGURE 98D

237/240

pMAB86

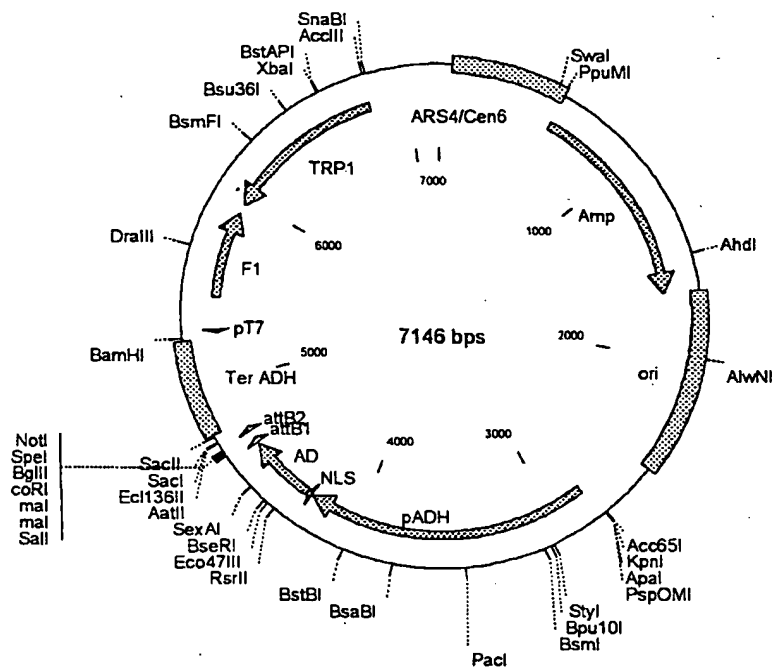


FIGURE 99A

pMAB86 7146 bp

GACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCATGATAATAATGGTTT
CTTAGGACGGATCGCTTGCTGTAACCTACACGCGCCTCGTATCTTTTAATGATGGAATA
ATTTGGGAATTTACTCTGTGTTTATTTATTTTTATGTTTTGTATTTGGATTTTAGAAAGT
AAATAAAGAAGGTAGAAGAGTTACGGAATGAAGAAAAAATAAACAAAGGTTTAAAAA
ATTTCAACAAAAGCGTACTTTACATATATATTTATTAGACAAGAAAAGCAGATTAAATA
GATATACATTTCGATTAACGATAAGTAAATGTAAATCACAGGATTTTCGTGTGTGGTCT
TCTACACAGACAAGATGAAACAATTCGGCATTAACTGAGAGCAGGAAGAGCAAGATA
AAAGGTAGTATTTGTTGGCGATCCCCCTAGAGTCTTTACATCTTCGGAACCAAAACT
ATTTTTCTTTAATTTCTTTTTTTTACTTTCTATTTTAAATTTATATATTTATATTAAAAA
ATTTAAATTATAATTATTTTTATAGCACGTGATGAAAAGGACCCAGGTGGCACTTTTCGG
GGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCG
CTCATGAGACAATAACCCCTGATAAATGCTTCAATAATATTGAAAAGGAAGAGTATGAGT
ATTCAACATTTCCGTGTGCGCCCTTATTCCCTTTTTTGCGGCATTTCCTGCTGTTTTT
GCTCACCAGAAACGCTGGTGAAAGTAAAGATGCTGAAGATCAGTTGGGTGCACGAGTG
GGTTACATCGAATGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAA
CGTTTCCAATGATGAGCACTTTTAAAGTCTGCTATGTGGCGCGGTATTATCCCGTATT
GACGCCGGGCAAGAGCAACTCGGTGCGGCATACACTATTCTCAGAATGACTTGGTTGAG
TACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGT
GCTGCCATAACCATGAGTGATAACACTGCGGCCAATTACTTCTGACAACGATCGGAGGA
CCGAAGGAGCTAACCGCTTTTTTTTCAACATGGGGGATCATGTAACCTCGCCTTGATCGT
TGGAACCGGAGCTGAATGAAGCCATACCAACGACGAGCGTGACACCACGATGCCTGTA
GCAATGGCAACAACGTTGCGCAAACTATTAAGTGGCGAACTACTTACTCTAGCTTCCCGG
CAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCC
CTTCCGGCTGGTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGT
ATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACG
GGCAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTG
ATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAAA
CTTCATTTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTTGATAATCTCATGACCAAA
ATCCCTTAACGTGAGTTTTCTGCTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGA
TCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAACAAAAAACCACCG
CTACCAGCGGTGGTTTGTGTTGCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAAC
GGCTTCAGCAGAGCGCAGATACCAATACTGTCTTCTAGTGAGCCGTAGTTAGGCCAC
CACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTG
GCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCG
GATAAGGCGCAGCGTCCGGCTGAACGGGGGGTTCGTGCACACAGCCAGCTTGGAGCGA
ACGACCTACACCGAACTGAGATACCTACAGCGTGAGCATTGAGAAAGCGCCACGCTTCCC
GAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACG
AGGAGCTTCCAGGGGGGAACGCCTGGTATCTTATAGTCCTGTGCGGTTTCGCCACCTC
TGACTTGAGCGTCGATTTTTTGTGATGCTCGTCAGGGGGGCGAGCCTATGGA AAAACGCC
AGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTT
CCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACC
GCTCGCCGACGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGC
CCAATACGCAAAACCGCCTCTCCCCGCGCGTTGGCCGATTCAATTAATGCAGCTGGCACGAC
AGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTACCTCACT
CATTAGGCACCCAGGCTTTACACTTTATGCTTCCGGCTCCTATGTTGTGTGGAATTTGTG
AGCGGATAACAATTTACACACAGGAAACAGCTATGACCATGATTACGCCAAGCTCGGAATT
AACCCTCACTAAAGGGAACAAAAGCTGGGTACCGGGCCCCCCTCGAGATCCGGGATCGA
AGAAATGATGGTAAATGAAATAGGAAATCAAGGAGCATGAAGGCAAAAGACAAATATAAG
GGTCGAACGAAAAATAAAGTGAAGTGTGATATGATGTATTTGGCTTTGCGGCGCCGA
AAAAACGAGTTTACGCAATTGCACAATCATGCTGACTCTGTGGCGGACCCGCGCTCTTGC
CGGCCCCGGCGATAACGCTGGGCGTGAGGCTGTGCCCGGCGGAGTTTTTTGCGCCTGCATT
TTCCAAGGTTTACCCTGCGCTAAGGGGCGAGATTGGAGAAGCAATAAGAATGCCGGTTGG
GGTTGCGATGACGACACGACCACTGGTGTCTATTATTAAAGTTGCCGAAAGAACCTG
AGTGCATTTGCAACATGAGTATACCTAGAAGAAATGAGCCAAGACTTGCGAGACGCGAGTTT
GCCGGTGGTGCGAACAATAGAGCGACCATGACCTTGAAGGTGAGACGCGCATAACCGCTA-

FIGURE 99B

GAGTACTTTGAAGAGGAAACAGCAATAGGGTTGCTACCAGTATAAATAGACAGGTACATA
CAACACTGGAAATGGTTGTCTGTTTGAGTACGCTTTCAATTCATTTGGGTGTGCACTTTA
TTATGTTACAATATGGAAGGGAACCTTACACTTCTCCTATGCACATATATTAATTAAGT
CCAATGCTAGTAGAGAAGGGGGTAACACCCCTCCGCGCTCTTTCCGATTTTTTTCTAA
ACCGTGGAATATTTCCGGATATCCTTTTGTGTTTCCGGGTGTACAATATGGACTTCCTCT
TTTCTGGCAACCAACCCATACATCGGGATTCTCTATAATACCTTCGTTGGTCTCCCTAAC
ATGTAGGTGGCGGAGGGGAGATATACAATAGAACAGATACCAGACAAGACATAATGGGCT
AAACAAGACTACACCAATTACACTGCCTCATTGATGGTGGTACATAACGAACATAACTG
TAGCCCTAGACTTGATAGCCATCATCATATCGAAGTTTCACTACCCCTTTTTCCATTTGCC
ATCTATTGAAGTAATAATAGGCGCATGCAACTTCTTTTCTTTTTTCTTTTCTCTCTC
CCCCGTTGTTGTCTCACCATATCCGCAATGACAAAAAATGATGGAAGACACTAAAGGA
AAAAATTAACGACAAAGACAGCACCAACAGATGTCGTTGTTCCAGAGCTGATGAGGGGTA
TCTTCGAACACACGAACTTTTTCTTCTTCAATTCACGCACACTACTCTCTAATGAGCA
ACGGTATACGGCCTTCTTCCAGTTACTTGAATTTGAAATAAAAAAAGTTTGCCGCTTG
CTATCAAGTATAAATAGACCTGCAATTATTAATCTTTTGTTCCTCGTCATTGTTCTCGT
TCCCTTTCTTCTTGTCTTTCTTTCTGCAATATTTCAAGCTATACCAAGCATACAATC
AACTCCAAGCTTATGCCCAAGAAGAAGCGGAAGGTCTCGAGCGGCGCAATTTTAATCAA
AGTGGGAATATGTGATAGCTCATTGTCTTCACTTTCACTAACAGTAGCAACGGTCCG
AACTCATACCAACTCAAAACAAATCTCAAGCGCTTTCACAACCAATTGCCTCCTTAAC
GTTTCATGATAACTTCATGAATAATGAAATCACGGCTAGTAAAAATTGATGATGGTAATAAT
TCAAAACCACTGTACCTGGTTGGACGGACCAAACTGCGTATAACGCGTTTGAATCACT
ACAGGGATGTTTAATACCACTACAATGGATGATGTATATACTATCTATTGATGATGAA
GATACCCACCAACCCAAAAAAGAGGGTGGGTGCGATCACAAGTTTGTACAAAAAAGCA
GGCTTGTGCGACCCCGGAATTGAGATCTACTAGTGCAGCGCCGACGCGTACCCAGCTTCT
TGTACAAAGTGTGACGTGAGCTCTAAGTAAGTAACGGCCGCCACCGCGGTGGAGCTTT
GGACTTCTTCGCCAGAGGTTTGGTCAAGTCTCCAATCAAGGTTGTGCGCTTGTCTACCTT
GCCAGAAATTTACGAAAAGATGGAAGGGTCAAATCGTTGGTAGATACGTTGTTGACAC
TTCTAAATAAGCGAATTTCTTATGATTTATGATTTTTATTATTAAATAAGTTATAAAAA
AATAAGTGTATACAAATTTTAAAGTGACTCTTAGGTTTAAAACGAAATTTCTGTTCTT
GAGTAACCTTTTCTGTAGGTCAGGTTGCTTTCTCAGGTATAGCATGAGGTGCGCTCTTAT
TGACCACACCTCTACCGGCATGCCGAGCAAATGCCTGCAAATCGCTCCCCATTTACCCCA
ATTGTAGATATGCTAACTCCAGCAATGAGTTGATGAATCTCGGTGTGTATTTTATGTCCT
CAGAGGACAATACCTGTTGTAATCGTTCTTCCACACGGATCCCAATTCGCCCTATAGTGA
GTCGTATTACCTCACTGGCCGTCGTTTTCACAACTGCTGACTGGGAAAACCTTGGCGT
TACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGA
GGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGACGCGCCC
TGTAGCGGCGCATTAAGCGCGCGGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTT
GCCAGCGCCCTAGCGCCCGCTCCTTTTCTGCTTTCTTCCCTTCTTTTCTCGCCACGTTCCGCC
GGCTTTCCCGCTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTA
CGGCACCTCGACCCCAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCC
TGATAGACGGTTTTTTCGCCCTTGTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTG
TTCCAAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTTTTGTATTATAAGGGATT
TTGCCGATTTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAAT
TTTAACAAAATATTAACGTTTACAATTTCTGATGCGGTATTTTCTCCTTACGCATCTGT
GCGGTATTTACACCGCAGGCAAGTGCACAAACAATACTTAAATAAATACTACTCAGTAA
TAACCTATTTCTTAGCATTTTGTACGAAATTTGCTATTTTGTAGAGTCTTTTACACCAT
TTGTCTCCACACCTCCGCTTACATCAACACCAATAACGCCATTTAATCTAAGCGCATCAC
CAACATTTTCTGGCGTCAGTCCACAGCTAACATAAAATGTAAGCTTTTCGGGGCTCTCTT
GCCTTCCAACCCAGTCAGAAATCGAGTTCCAATCCAAAAGTTACCTGTCCCACCTGCTT
CTGAATCAAACAAGGAATAAACGAATGAGGTTTCTGTGAAGCTGCACTGAGTAGTATGT
TGCAGTCTTTTGGAAATACGAGTCTTTAATAACTGGCAAACCGAGGAACCTTTGGTATT
CTTGCCACGACTCATCTCCATGCAGTTGGACGATATCAATGCCGTAATCATTGACCAGAG
CCAAAACATCCTCCTTAGGTTGATTACGAAACACGCCAACCAAGTATTTTCGGAGTGCCTG
AACTATTTTTATATGCTTTTACAAGACTTGAAATTTTCTTGAATAACCGGGTCAATTG
TTCTCTTTCTATTGTTGGCACACATATAATACCCAGCAAGTCAGCATCGGAATCTAGAGCAC
ATTCTGCGGCTCTGTGCTCTGCAAGCCGCAAACTTTACCAATGGACCAGAACTCTG
TGAAATTAATAACAGACATACTCCAAGCTGCCTTTGTGTGCTTAATCACGTATACTCACG
TGCTCAATAGTCACCAATGCCCTCCCTCTTGGCCCTCTCCTTTTCTTTTTTCGACCGAAT-

TAATTCTTAATCGGCAAAAAAAGAAAAGCTCCGGATCAAGATTGTACGTAAGGTGACAAG
CTATTTTTCAATAAAGAATATCTTCCACTACTGCCATCTGGCGTCATAACTGCAAAGTAC
ACATATATTACGATGCTGTCTATTAAATGCTTCCTATATTATATATATAGTAATGTCGTT
TATGGTGCACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGCCCCGACACC
CGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAGAC
AAGCTGTGACCGTCTCCGGGAGCTGCATGTGTGAGAGGTTTTACCGTCATCACCGAAC
GCGCGA

FIGURE 99D

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM
(PCT Rule 13bis)

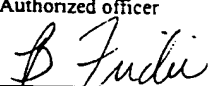
SM
REC'D

A. The indications made below relate to the microorganism referred to in the description on page <u>54</u> , line <u>8</u> .	
B. IDENTIFICATION OF DEPOSIT	
Further deposits are identified on an additional sheet <input checked="" type="checkbox"/>	
Name of depositary institution Agricultural Research Culture Collection (NRRL) International Depository Authority	
Address of depositary institution (including postal code and country) 1815 N. University Street Peoria, Illinois 61604 United States of America	
Date of deposit February 27, 1999	Accession Number NRRL B-30103
C. ADDITIONAL INDICATIONS (leave blank if not applicable)	
This information is continued on an additional sheet <input type="checkbox"/>	
Escherichia coli DB3.1(pEZC15101)	
In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")	

<p style="text-align: center;">For receiving Office use only</p> <p><input checked="" type="checkbox"/> This sheet was received with the international application</p> <p>Authorized officer <i>B. Indici</i></p>	<p style="text-align: center;">For International Bureau use only</p> <p><input type="checkbox"/> This sheet was received by the International Bureau on:</p> <p>Authorized officer</p>
---	--

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM
(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>55</u> , line <u>16</u> .	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input checked="" type="checkbox"/>	
Name of depositary institution Agricultural Research Culture Collection (NRRL) International Depository Authority	
Address of depositary institution (including postal code and country) 1815 N. University Street Peoria, Illinois 61604 United States of America	
Date of deposit February 27, 1999	Accession Number NRRL B-30100
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
Escherichia coli DB3.1(pENTR-1A) In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")	

For receiving Office use only	For International Bureau use only
<input checked="" type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer 	Authorized officer

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM
(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>55</u> , line <u>16</u> .	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input checked="" type="checkbox"/>	
Name of depositary institution Agricultural Research Culture Collection (NRRL) International Depository Authority	
Address of depositary institution (including postal code and country) 1815 N. University Street Peoria, Illinois 61604 United States of America	
Date of deposit February 27, 1999	Accession Number NRRL B-30102
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
Escherichia coli DB3.1(pENTR-3C) In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")	

<p style="text-align: center;">For receiving Office use only</p> <p><input checked="" type="checkbox"/> This sheet was received with the international application</p> <p>Authorized officer <i>B. Judie</i></p>	<p style="text-align: center;">For International Bureau use only</p> <p><input type="checkbox"/> This sheet was received by the International Bureau on:</p> <p>Authorized officer</p>
--	--

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM
(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>55</u> , line <u>16</u> .	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input checked="" type="checkbox"/>	
Name of depositary institution Agricultural Research Culture Collection (NRRL) International Depository Authority	
Address of depositary institution (including postal code and country) 1815 N. University Street Peoria, Illinois 61604 United States of America	
Date of deposit February 27, 1999	Accession Number NRRL B-30101
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
Escherichia coli DB3.1(pENTR-2B) In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")	

<p style="text-align: center;">For receiving Office use only</p> <div style="border: 1px solid black; padding: 5px; height: 40px;"> <input checked="" type="checkbox"/> This sheet was received with the international application </div> <div style="border: 1px solid black; padding: 5px; height: 40px;"> Authorized officer </div>	<p style="text-align: center;">For International Bureau use only</p> <div style="border: 1px solid black; padding: 5px; height: 40px;"> <input type="checkbox"/> This sheet was received by the International Bureau on: </div> <div style="border: 1px solid black; padding: 5px; height: 40px;"> Authorized officer </div>
---	--

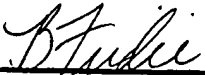
INDICATIONS RELATING TO A DEPOSITED MICROORGANISM
(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>WIPO</u> <u>PCT</u> <u>20-21</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input checked="" type="checkbox"/>	
Name of depositary institution Agricultural Research Culture Collection (NRRL) International Depository Authority	
Address of depositary institution (including postal code and country) 1815 N. University Street Peoria, Illinois 61604 United States of America	
Date of deposit February 27, 1999	Accession Number NRRL B-30108
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
Escherichia coli DB10B(pCMVSPORT6) In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")	

For receiving Office use only	For International Bureau use only
<input checked="" type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer 	Authorized officer

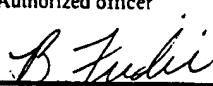
INDICATIONS RELATING TO A DEPOSITED MICROORGANISM
(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>54</u> , line <u>9</u> .	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input checked="" type="checkbox"/>	
Name of depositary institution Agricultural Research Culture Collection (NRRL) International Depository Authority	
Address of depositary institution (including postal code and country) 1815 N. University Street Peoria, Illinois 61604 United States of America	
Date of deposit February 27, 1999	Accession Number NRRL B-30105
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
Escherichia coli DB3.1(pEZC15103) In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")	

For receiving Office use only	For International Bureau use only
<input checked="" type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer 	Authorized officer

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM
(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>54</u> , line <u>9</u> .	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input checked="" type="checkbox"/>	
Name of depositary institution Agricultural Research Culture Collection (NRRL) International Depository Authority	
Address of depositary institution (including postal code and country) 1815 N. University Street Peoria, Illinois 61604 United States of America	
Date of deposit February 27, 1999	Accession Number NRRL B-30104
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
Escherichia coli DB3.1(pEZC15102) In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")	

For receiving Office use only	For International Bureau use only
<input checked="" type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer 	Authorized officer

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM
(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>52</u> , line <u>31</u> .	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input checked="" type="checkbox"/>	
Name of depositary institution Agricultural Research Culture Collection (NRRL) International Depository Authority	
Address of depositary institution (including postal code and country) 1815 N. University Street Peoria, Illinois 61604 United States of America	
Date of deposit February 27, 1999	Accession Number NRRL B-30099
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
Escherichia coli DB3.1(pAHPKan) or Escherichia coli DB3.1(pAttPKan) In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit") 	

<p align="center">For receiving Office use only</p> <p><input checked="" type="checkbox"/> This sheet was received with the international application</p> <p>Authorized officer Barbara Fridie PCT Operations - IPD Team 1 703/305-3147 703/305-3230 (FAX)</p>	<p align="center">For International Bureau use only</p> <p><input type="checkbox"/> This sheet was received by the International Bureau on:</p> <p>Authorized officer</p>
---	---

Escherichia coli DB3.1(pENTR-3C)

ICELAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the Icelandic Patent Office), or has been finally decided upon by the Icelandic Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in Rule 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

NORWAY

The applicant hereby requests that, until the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Norwegian Patent office or any person approved by the applicant in the individual case.

SINGAPORE

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

Escherichia coli DB3.1(pENTR-3C)**SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent office or any person approved by the applicant in the individual case.

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

Escherichia coli DB3.1(pENTR-2B)**AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

CANADA

The applicant hereby requests that, until either a Canadian patent has been issued on the basis of the application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the furnishing of a sample of deposited biological material referred to in the application only be effected to an independent expert nominated by the Commissioner of Patents.

DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent office or any person approved by the applicant in the individual case.

FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Registration), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the National Board of Patents and Registration or any person approved by the applicant in the individual case.

Escherichia coli DB3.1(pENTR-2B)**ICELAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Icelandic Patent Office), or has been finally decided upon by the Icelandic Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in Rule 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

NORWAY

The applicant hereby requests that, until the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Norwegian Patent office or any person approved by the applicant in the individual case.

SINGAPORE

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

Escherichia coli DB3.1(pENTR-2B)

SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent office or any person approved by the applicant in the individual case.

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

Escherichia coli DB3.1(pENTR-1A)**AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

CANADA

The applicant hereby requests that, until either a Canadian patent has been issued on the basis of the application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the furnishing of a sample of deposited biological material referred to in the application only be effected to an independent expert nominated by the Commissioner of Patents.

DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent office or any person approved by the applicant in the individual case.

FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Registration), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the National Board of Patents and Registration or any person approved by the applicant in the individual case.

Escherichia coli DB3.1(pENTR-1A)**ICELAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Icelandic Patent Office), or has been finally decided upon by the Icelandic Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in Rule 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

NORWAY

The applicant hereby requests that, until the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Norwegian Patent office or any person approved by the applicant in the individual case.

SINGAPORE

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

Escherichia coli DB3.1(pENTR-1A)

SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent office or any person approved by the applicant in the individual case.

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

*Escherichia coli DB10B(pCMVSPORT6)***AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

CANADA

The applicant hereby requests that, until either a Canadian patent has been issued on the basis of the application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the furnishing of a sample of deposited biological material referred to in the application only be effected to an independent expert nominated by the Commissioner of Patents.

DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent office or any person approved by the applicant in the individual case.

FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Registration), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the National Board of Patents and Registration or any person approved by the applicant in the individual case.

Escherichia coli DB3.1(pAHPKan) or *Escherichia coli* DB3.1(pAttPKan)

AUSTRALIA

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

CANADA

The applicant hereby requests that, until either a Canadian patent has been issued on the basis of the application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the furnishing of a sample of deposited biological material referred to in the application only be effected to an independent expert nominated by the Commissioner of Patents.

DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent office or any person approved by the applicant in the individual case.

FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Registration), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the National Board of Patents and Registration or any person approved by the applicant in the individual case.

Escherichia coli DB3.1(pAHPKan) or *Escherichia coli* DB3.1(pAttPKan)

ICELAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the Icelandic Patent Office), or has been finally decided upon by the Icelandic Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in Rule 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

NORWAY

The applicant hereby requests that, until the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Norwegian Patent office or any person approved by the applicant in the individual case.

SINGAPORE

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

Escherichia coli DB3.1(pAHPKan) or *Escherichia coli* DB3.1(pAttPKan)

SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent office or any person approved by the applicant in the individual case.

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

*Escherichia coli DB10B(pCMVSPORT6)***ICELAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Icelandic Patent Office), or has been finally decided upon by the Icelandic Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in Rule 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

NORWAY

The applicant hereby requests that, until the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Norwegian Patent office or any person approved by the applicant in the individual case.

SINGAPORE

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

Escherichia coli DB10B(pCMVSPORT6)

SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent office or any person approved by the applicant in the individual case.

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

Escherichia coli DB3.1(pEZC15103)**AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

CANADA

The applicant hereby requests that, until either a Canadian patent has been issued on the basis of the application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the furnishing of a sample of deposited biological material referred to in the application only be effected to an independent expert nominated by the Commissioner of Patents.

DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent office or any person approved by the applicant in the individual case.

FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Registration), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the National Board of Patents and Registration or any person approved by the applicant in the individual case.

Escherichia coli DB3.1(pEZC15103)

ICELAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the Icelandic Patent Office), or has been finally decided upon by the Icelandic Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in Rule 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

NORWAY

The applicant hereby requests that, until the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Norwegian Patent office or any person approved by the applicant in the individual case.

SINGAPORE

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

Escherichia coli DB3.1(pEZC15103)**SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent office or any person approved by the applicant in the individual case.

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

Escherichia coli DB3.1(pEZC15102)**AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

CANADA

The applicant hereby requests that, until either a Canadian patent has been issued on the basis of the application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the furnishing of a sample of deposited biological material referred to in the application only be effected to an independent expert nominated by the Commissioner of Patents.

DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent office or any person approved by the applicant in the individual case.

FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Registration), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the National Board of Patents and Registration or any person approved by the applicant in the individual case.

Escherichia coli DB3.1(pEZC15102)

ICELAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the Icelandic Patent Office), or has been finally decided upon by the Icelandic Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in Rule 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

NORWAY

The applicant hereby requests that, until the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Norwegian Patent office or any person approved by the applicant in the individual case.

SINGAPORE

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

Escherichia coli DB3.1(pEZC15102)**SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent office or any person approved by the applicant in the individual case.

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

Escherichia coli DB3.1(pEZC15101)**AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

CANADA

The applicant hereby requests that, until either a Canadian patent has been issued on the basis of the application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the furnishing of a sample of deposited biological material referred to in the application only be effected to an independent expert nominated by the Commissioner of Patents.

DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent office or any person approved by the applicant in the individual case.

FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Registration), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the National Board of Patents and Registration or any person approved by the applicant in the individual case.

Escherichia coli DB3.1(pEZC15101)**ICELAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Icelandic Patent Office), or has been finally decided upon by the Icelandic Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in Rule 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

NORWAY

The applicant hereby requests that, until the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Norwegian Patent office or any person approved by the applicant in the individual case.

SINGAPORE

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

Escherichia coli DB3.1(pEZC15101)

SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent office or any person approved by the applicant in the individual case.

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

Escherichia coli DB3.1(pENTR-3C)**AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

CANADA

The applicant hereby requests that, until either a Canadian patent has been issued on the basis of the application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the furnishing of a sample of deposited biological material referred to in the application only be effected to an independent expert nominated by the Commissioner of Patents.

DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent office or any person approved by the applicant in the individual case.

FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Registration), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the National Board of Patents and Registration or any person approved by the applicant in the individual case.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/05432

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : Please See Extra Sheet.
US CL : 435/91.2, 252.3, 320.1; 530/350; 536/ 23.1, 24.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/91.2, 252.3, 320.1; 530/350; 536/ 23.1, 24.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P ---- Y,P	US 5,888,732 A (HARTLEY et al.) 30 March 1999, see entire document.	1-21, 25-30 36-38 ----- 22-24, 31-35
X - Y	HASAN et al. Escherichia coli genome targeting, I. Cre-lox-mediated in vitro generation of ori- plasmids and their in vivo chromosomal integration and retrieval. Gene. 1994, Vol. 150, pages 51-56, see entire document.	1-5, 10, 11, 19-21 ----- 15-18, 22-38
X - Y	KATZ et al. Site-specific recombination in Escherichia coli between the att sites of plasmid pSE211 from Saccharopolyspora erythraea. Mol. Gen. Genet. 1991, Vol. 227, pages 155-159, see entire document.	1-11, 19-21 ----- 15-18, 22-38

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 08 MAY 2000	Date of mailing of the international search report 23 MAY 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer <i>Irem Yucel</i> IREM YUCEL Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/05432

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X - Y	ASTUMIAN et al. Site-specific recombination between cloned attP and attB sites from the Haemophilus influenzae bacteriophage HP1 propagated in recombination deficient Escherichia coli. J of Bacteriology. March 1989, Vol. 171, No. 3, pages 1747-1750, see entire document.	1-11, 19-21 ----- 15-18, 22-38

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/05432

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (7):

C07H 21/04; C07K 1/00, 14/00; C12N 1/21, 15/00, 15/09, 15/63, 15/70; C12P 19/34

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

WEST, STN (CAPLUS); DIALOG (MEDLINE, BIOSIS, SCISEARCH, PASCAL)

Terms: att (B?, P?, R?, L?), MCS, POLYLINKER, PLASMID, VECTOR, LOCALIZATION, SIGNAL, TRANSCRIPTION, TERMIN?, TRANSLATION?, ORI, REPLICON, GST, HEXHIST?, THIOREDOX?, CLEAVAGE, SITE?, SPECIF?, DIRECT?, RECOMBIN?, CLON?, INSERT?

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☒ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☒ FADED TEXT OR DRAWING
- ☐ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.